PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/37, C07K 14/025

A1

(11) International Publication Number: WO 99/02694

(43) International Publication Date: 21 January 1999 (21.01.99)

(21) International Application Number: PCT/AU98/00530

(22) International Filing Date: 9 July 1998 (09.07.98)

(30) Priority Data:

PO 7765	9 July 1997 (09.07.97)	AU
PO 9467	11 September 1997 (11.09.97)	AU

(71) Applicant *(for all designated States except US)*: THE UNIVERSITY OF QUEENSLAND [AU/AU]; St. Lucia, QLD 4072 (AU).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: FRAZER, Ian [AU/AU]; 64 Eighth Avenue, St. Lucia, QLD 4067 (AU). ZHOU, Jian [AU/AU]; 21 Cooranga Street, Jindalee, QLD 4074 (AU).

(74) Agent: HORSBURGH, Mark, Alexander; Fisher Adams Kelly, Level 13, 10 Eagle Street, Brisbane, QLD 4000 (AU).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: NUCLEIC ACID SEQUENCE AND METHOD FOR SELECTIVELY EXPRESSING A PROTEIN IN A TARGET CELL OR TISSUE

(57) Abstract

A synthetic nucleic acid sequence and a method are disclosed for selectively expressing a protein in a target cell or tissue of a mammal. Selective expression is effected by replacing at least one existing codon of a parent nucleic acid sequence encoding a protein of interest with a synonymous codon to produce a synthetic nucleic acid sequence having altered translational kinetics compared to the parent nucleic acid sequence. The synonymous codon is selected such that it corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to one or more other cells or tissues of the mammal.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

TITLE

"NUCLEIC ACID SEQUENCE AND METHOD FOR SELECTIVELY EXPRESSING A PROTEIN IN A TARGET CELL OR TISSUE"

FIELD OF THE INVENTION

THIS INVENTION relates generally to gene therapy. More particularly, the present invention relates to a synthetic nucleic acid sequence and to a method for selectively expressing a protein in a target cell or tissue in which at least one existing codon of a parent nucleic acid sequence encoding the protein has been replaced with a synonymous codon. The invention also relates to production of virus particles using one or more synthetic nucleic acid sequences and the method according to the invention.

BACKGROUND OF THE INVENTION

While gene therapy is of great clinical interest for treatment of gene defects, this therapy has not entered into mainstream clinical practice because selective delivery of genes to target tissues has proven extremely difficult. Currently, viral vectors are used, particularly retroviruses and adenovirus, which are to some extent selective. However, many vector systems are by their nature unable to produce stable integrants and some also invoke immune responses thereby preventing effective treatment. Alternatively, "naked" DNA is packaged in liposomes or other similar delivery systems. A major

problem to be overcome is that such gene delivery systems themselves are not tissue selective, whereas selective targeting of genes to particular tissues would be desirable for many disorders (*e.g.*, cancer therapy). While use of tissue specific promoters to target gene therapy has been effective in some animal models it has proven less so in man, and selective tissue specific promoters are not available for a wide range of tissues.

The current invention has arisen unexpectedly from recent investigations exploring why papillomavirus (PV) late gene expression is restricted to differentiated keratinocytes. In this regard, it is known that PV late genes L1 and L2 are only expressed in non-dividing differentiated keratinocytes (KCs). Many investigators including the present inventors have been unable to detect significant PV L1 and L2 protein expression when these genes are transduced or transfected into undifferentiated cultured cells, using a range of conventional constitutive viral promoters including retroviral long terminal repeats (LTRs) and the strong constitutive promoters of CMV and SV40.

PV L1 mRNA can however be efficiently translated *in vitro* using rabbit reticulocyte cell lysate, suggesting that there are no cellular inhibitors in the lysate interfering with translation of L1. The major difference between the *in vitro* and *in vivo* translation systems is that L1 comprises the dominant L1 mRNA in *in vitro* translation reactions, while it constitutes a minor fraction among the cellular mRNAs in intact cells.

In vivo, PV late proteins are not produced in undifferentiated KC. However, they are expressed in large quantity in highly differentiated KC. The mechanism of this tight control of late gene expression has been poorly understood, and searches by many groups for KC specific PV gene transcriptional control proteins have been unrewarding.

Blockage to translation of L1 mRNA *in vivo* has been attributed to sequences within the L1 ORF (Tan *et al.* 1995, *J. Virol.* **69** 5607-5620; Tan and Schwartz, 1995, *J. Virol.* **69** 2932-2945). By using a Rev and Rev-responsive element of HIV, such inhibition could be overcome (Tan *et al.* 1995, *supra*). Accordingly, the inventors examined whether removal of putative "inhibitory sequences" in the L1 ORF would allow production of L1 protein in undifferentiated cells. Deletion mutagenesis of BPV L1 to remove putative inhibitory sequences and expression of resultant deletion mutants in CV-1 cells revealed surprisingly that despite expression of L1 mRNA, L1 protein could not be detected.

In view of the foregoing, it has been difficult hitherto to understand how papillomaviruses produce large amounts of L1 protein in the late stage of their life cycle using this apparently "untranslatable" gene.

Surprisingly, however, it has now been discovered that PV L1 protein can be produced at substantially enhanced levels in an undifferentiated host cell by replacing existing codons of a native L1 gene with synonymous codons used at relatively high frequency by genes of the undifferentiated host cell

compared to the existing codons. It has also been found unexpectedly that there are substantial differences in the relative abundance of particular isoaccepting transfer RNAs (tRNAs) in different cells or tissues and this plays a pivotal role in protein expression from a gene with a given codon usage or composition. This discovery has been reduced to practice in synthetic nucleic acid sequences and generic methods, which utilize codon alteration as a means for targeting expression of a protein to particular cells or tissues or alternatively, to cells in a specific state of differentiation.

OBJECT OF THE INVENTION

It is therefore an object of the present invention to provide a synthetic nucleic acid sequence and a method for selectively expressing a protein in a target cell or tissue which sequence and method ameliorate at least some of the disadvantages associated with the prior art.

SUMMARY OF THE INVENTION

Accordingly, in one aspect of the invention, there is provided a synthetic nucleic acid sequence capable of selectively expressing a protein in a target cell or tissue of a mammal, wherein said selective expression is effected by replacing at least one existing codon of a parent nucleic acid sequence with a synonymous codon to form said synthetic nucleic acid sequence.

Suitably, said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to one or more other cells or tissues of the mammal.

Preferably, said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to a precursor cell or tissue.

Alternatively, said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to a cell or tissue derived therefrom.

Advantageously, said corresponding iso-tRNA in said target cell or tissue is at a level which is at least 110%, preferably at least 200%, more preferably at least 500%, and most preferably at least 1000%, of that expressed in the or each other cell or tissue of the mammal.

Alternatively, the synonymous codon may be selected from the group consisting of (1) a codon used at relatively high frequency by genes, preferably highly expressed genes, of the target cell or tissue, (2) a codon used at relatively high frequency by genes, preferably highly expressed genes, of the or each other cell or tissue, (3) a codon used at relatively high frequency by genes, preferably highly expressed genes, of the mammal, (4) a codon used at relatively low frequency by genes of the target cell

or tissue, (5) a codon used at relatively low frequency by genes of the or each other cell or tissue, (6) a codon used at relatively low frequency by genes of the mammal, (7) a codon used at relatively high frequency by genes of another organism, and (8) a codon used at relatively low frequency by genes of another organism.

In a preferred embodiment, the at least one existing codon and the synonymous codon are preferably selected such that said protein is expressed from said synthetic nucleic acid sequence in said target cell or tissue at a level which is at least 110%, preferably at least 200%, more preferably at least 500%, and most preferably at least 1000%, of that expressed from said parent nucleic acid sequence in said target cell or tissue.

In another aspect, the invention resides in a method for selectively expressing a protein in a target cell or tissue of a mammal, wherein said selective expression is effected by replacing at least one existing codon of a parent nucleic acid sequence with a synonymous codon to form said synthetic nucleic acid sequence.

Preferably, the method is further characterized by the steps of:

(a) replacing at least one existing codon of a parent nucleic acid sequence encoding said protein with a synonymous codon to produce a synthetic nucleic acid sequence having altered translational kinetics compared to said parent nucleic acid sequence such that said protein is selectively expressible in said target cell or tissue;

(b) administering to the mammal and introducing into said target cell or tissue, or a precursor cell or precursor tissue thereof, said synthetic nucleic acid sequence operably linked to one or more regulatory nucleotide sequences; and

(c) selectively expressing said protein in said target cell or tissue.

Preferably, the method further includes, prior to step (a):

(i) measuring relative abundance of different isoacceptor transfer RNAs in said target cell or tissue, and in one or more other cells or tissues of the mammal; and

(ii) identifying said at least one existing codon and said synonymous codon based on said measurement, wherein said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the existing codon, is in higher abundance in said target cell or tissue relative to the or each other cell or tissue of the mammal.

Suitably, step (ii) above is further characterized in that said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to a precursor cell or tissue.

Alternatively, step (ii) above is further characterized in that said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to a cell or tissue derived therefrom.

Alternatively, the method further includes, prior to step (a), identifying said at least one existing codon and said synonymous codon based on respective relative frequencies of particular codons used by genes selected from the group consisting of (I) genes of the target cell or tissue, (II) genes of the or each other cell or tissue, (III) genes of the mammal, and (IV) genes of another organism.

In yet another aspect, the invention provides a method for expressing a protein in a target cell or tissue from a first nucleic acid sequence including the steps of:

introducing into said target cell or tissue, or a precursor cell or precursor tissue thereof, a second nucleic acid sequence encoding at least one isoaccepting transfer RNA wherein said second nucleic acid sequence is operably linked to one or more regulatory nucleotide sequences, and wherein said at least one isoaccepting transfer RNA is normally in relatively low abundance in said target cell or tissue and corresponds to a codon of said first nucleic acid sequence.

In a further aspect, the invention extends to a method for producing a virus particle in a cycling eukaryotic cell, said virus particle comprising at least one protein necessary for assembly of said virus particle, wherein said at least one protein is not expressed in said cell from a parent nucleic acid sequence at a level sufficient to permit virus assembly therein, said method including the steps of:

(a) replacing at least one existing codon of said parent nucleic acid sequence with a synonymous codon to produce a synthetic nucleic acid sequence having altered translational kinetics compared to said parent nucleic acid sequence such that said at least one protein is expressible from said synthetic nucleic acid sequence in said cell at a level sufficient to permit virus assembly therein;

(b) introducing into said cell or a precursor thereof said synthetic nucleic acid sequence operably linked to one or more regulatory nucleotide sequences; and

(c) expressing said at least one protein in said cell in the presence of other viral proteins required for assembly of said virus particle to thereby produce said virus particle.

In yet a further aspect of the invention, there is provided a method for producing a virus particle in a cycling cell, said virus particle comprising at least one protein necessary for assembly of said virus particle, wherein said at least one protein is not expressed in said cell from a parent nucleic acid sequence at a level sufficient to permit virus assembly therein, and wherein at least one existing codon of said parent nucleic acid sequence is rate limiting for the production said at least one protein to said level, said method including the step of introducing into said cell a nucleic acid sequence capable of expressing therein an isoaccepting transfer RNA specific for said at least one codon.

BRIEF DESCRITPION OF THE DRAWINGS

Figure 1A depicts the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of BPV1 L1. Amino acids (in single letter code) are presented below the second nucleotide of each codon. Mutations introduced into the genes are indicated above the corresponding nucleotides of the original sequence. Horizontal lines indicate the sites and enzymes used for cloning. This replacement of nucleotides resulted in a nucleic acid sequence encoding BPV-1 L1 polypeptide with an amino acid sequences identical to the wild type, but having synonymous codons that are frequently used by mammalian genes.

Figure 1B shows the nucleotide sequence (SEQ ID NO:5) and deduced amino acid sequence (SEQ ID NO:6) relating to BPV1 L2 ORF. Amino acids (in single letter code) are presented below the second nucleotide of each codon. Mutations introduced into the genes are indicated above the corresponding nucleotides of the original sequence. Horizontal lines indicate the sites and enzymes used for cloning. This replacement of nucleotides resulted in a nucleic acid sequence encoding BPV-1 L2 polypeptide with an amino acid sequences identical to the wild type, but having synonymous codons that are frequently used by mammalian genes.

Figure 1C depicts the nucleotide sequence (SEQ ID NO:9) and deduced amino acid sequence (SEQ ID NO:10) of green fluorescent protein (GFP). Amino acids (in single letter code) are presented below the

second nucleotide of each codon. Mutations introduced into the genes are indicated above the corresponding nucleotides of the original sequence. Horizontal lines indicate the sites and enzymes used for cloning. This replacement of nucleotides resulted in a nucleic acid sequence encoding GFP polypeptide with an amino acid sequence identical to the native sequence modified for optimal expression in eukaryotic cells, but having synonymous codons that are frequently used by papillomavirus genes.

Figure 2A shows detection of L1 protein expressed from synthetic and wild type BPV1 L1 genes. Cos-1 cells were transfected with a synthetic L1 expression plasmid pCDNA/HBL1, and a wild type L1 expression plasmid pCDNA/BPVL1wt. The expression of L1 was detected by immunofluorescent staining. Cells were fixed after 36 hrs and incubated with rabbit anti-BPV1 L1 antiserum, followed by FITC-conjugated goat anti-rabbit IgG antibody.

Figure 2B shows detection by Western blot of L1 protein from Cos-1 cells transfected with pCDNA/HBL1 and pCDNA/BPVL1wt.

Figure 2C shows a Northern blot in which L1 mRNA extracted from transfected cells was probed with ^{32}P-labeled probes produced from wild type L1 sequence. The amount of mRNA loaded in respective lanes was examined by hybridization of the mRNA sample with a *gapdh* probe.

Figure 3A shows detection of L2 protein expressed from synthetic and wild type BPV1 L2 genes. Cos-1 cells were transfected with a synthetic L2 expression plasmid pCDNA/HBL2, and a wild type L2

expression plasmid pCDNA/BPVL2wt. The expression of L2 was detected by immunofluorescent staining. Cells were fixed after 36 hrs and incubated with rabbit anti-BPV1 L2 antiserum, followed by FITC-conjugated goat anti-rabbit IgG antibody.

Figure 3B shows detection by Western blot of L2 protein from Cos-1 cells transfected with pCDNA/HBL2 and pCDNA/BPVL2wt.

Figure 3C shows a Northern blot in which L2 mRNA extracted from transfected cells was probed with ^{32}P-labeled probes produced from wild type L2 sequence. The amount of mRNA loaded in respective lanes was examined by hybridization of the mRNA sample with a *gapdh* probe.

Figure 4 shows *in vitro* translation of BPVL1 sequences, wild type BPVL1 (wt) or synthetic L1 (HB) using rabbit reticulocyte lysate or wheat germ extract in the presence of ^{35}S-methionine. In the top panel, wt L1 or HB L1 plasmid DNA was added to the T7 DNA polymerase-coupled *in vitro* translation system. L1 protein was detected by Western blot analysis. In the bottom panel, the translation efficiency of wt L1 or HB L1 sequences in the presence or absence of tRNA was compared. Translation was carried out in rabbit reticulocyte lysate (rabbit) or wheat germ extract (wheat), and samples were collected every two minutes starting from minute 8. Left side of lower panel indicates if 10^{-5} M bovine liver or yeast tRNA was supplied.

Figure 5A is a schematic representation of plasmids used to determine L2 expression from BPV cryptic promoter(s). The wild type L1 sequence and

most of the wild type L2 sequence were deleted from the BPV1 genome by *Bam*HI and *Hind*III digestion and the remaining BPV1 sequence (in yellow) was cloned into pUC18. Wild type or synthetic humanized L2 sequences (in red) were inserted into the *Bam*HI site of the BPV1 genome. The position of the inserted SV40 *ori* sequence (in white) is indicated. The plasmid in which modified L2 was used but without SV40 *ori* sequence was also used as a control. The plasmids were transfected into Cos-1 cells and the expression of L2 protein was determined using BPV1 L2-specific polyclonal antiserum followed by FITC-linked anti rabbit IgG.

Figure 5B shows expression of L2 protein from native papillomavirus promoter. The plasmids shown in Figure 5A were used to transfect Cos-1 cells and the expression of L2 protein was determined using BPV1 L2-specific polyclonal antiserum followed by FITC-linked anti rabbit IgG. A mock transfection in which the cells did not receive plasmid was used as control.

Figure 6 shows expression of GFP in Cos-1 cells transfected with wild-type *gfp* (wt) or a synthetic *gfp* gene carrying codons used at relatively high frequency by papillomavirus genes (p). The mRNA extracted from cells transfected with *gfp* or P *gfp* was probed with ^{32}P-labeled *gfp* probe and is shown on the right panel, using *gapdh* as a reference gene.

Figure 7 shows the expression pattern of GFP *in vivo* from wild-type *gfp* gene, or a synthetic *gfp* gene carrying codons used at relatively high frequency by papillomavirus genes. Using a gene gun,

mice were shot with PGFP (left panel) and GFP (right panel) expression plasmids encoding GFP protein. A transverse section of the mouse skin section shows where the *gfp* gene is expressed. Bright-field photographs of the same section where dermis (D) epidermis (E) are highlighted are shown to identify the location of fluorescence in the epidermis. Arrows indicate fluorescent signals.

DETAILED DESCRIPTION

The present invention arises from the unexpected discovery that the relative abundance of different isoaccepting transfer RNAs varies in different cells or tissues, or alternatively in cells or tissues in different states of differentiation or in different stages of the cell cycle, and that such differences may be exploited together with codon composition of a gene to regulate and direct expression of a protein to a particular cell or tissue, or alternatively to a cell or tissue in a specific state of differentiation or in a specific stage of the cell cycle. According to the present invention, this selective targeting is effected by replacing at least one existing codon of a parent nucleic acid sequence encoding the protein with a synonymous codon.

Replacement of synonymous codons for existing codons is not new *per se*. In this regard, we refer to International Application Publication No WO 96/09378 which utilizes such substitution to provide a method of expressing proteins of eukaryotic and viral

origin at high levels in *in vitro* mammalian cell culture systems, the main thrust of the method being the harvesting of such proteins. In distinct contrast, the present invention utilizes substitution of one or more codons in a gene for targeting expression of the gene to particular cells or tissues with the ultimate aim of facilitating gene therapy as described herein.

The term "synonymous codon" as used herein refers to a codon having a different nucleotide sequence to an existing codon but encoding the same amino acid as the existing codon.

By "isoaccepting transfer RNA" is meant one or more transfer RNA molecules that differ in their anticodon structure but are specific for the same amino acid.

Throughout this specification, unless the context requires otherwise, the words "comprise", comprises" and "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Selection of synonymous codons

Determination of relative abundance of different tRNA species in different cells

Advantageously, the synonymous codon corresponds to an iso-tRNA (iso-tRNA) which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the

target cell or tissue relative to one or more other cells or tissues of the mammal.

Any method for determining the relative abundance of an iso-tRNA in two or more cells or tissues may be employed. For example, such method may include isolating two or more particular cells or tissues from a mammal, preparing an RNA extract from each cell or tissue which extract includes tRNA, and probing each extract respectively with different nucleic acid sequences each being specific for a particular iso-tRNA to determine the relative abundance of an iso-tRNA between the two or more cells or tissues.

Suitable methods for isolating particular cells or tissues are well known to those of skill in the art. For example, one can take advantage of one or more particular characteristics of a cell or tissue to specifically isolate the cell or tissue from a heterogeneous population. Such characteristics include, but are not limited to, anatomical location of a tissue, cell density, cell size, cell morphology, cellular metabolic activity, cell uptake of ions such as Ca^{2+}, K^{+}, and H^{+} ions, cell uptake of compounds such as stains, markers expressed on the cell surface, cytokine expression, protein fluorescence, and membrane potential. Suitable methods that may be used in this regard include surgical removal of tissue, flow cytometry techniques such as fluorescence-activated cell sorting (FACS), immunoaffinity separation (*e.g.*, magnetic bead separation such as Dynabead™ separation), density separation (*e.g.*, metrizamide, Percoll™, or Ficoll™ gradient

centrifugation), and cell-type specific density separation (*e.g.*, Lymphoprep™). For example, dividing cells or blast cells may be separated from non-dividing cells or resting cells according to cell size by FACS or metrizamide gradient separation.

Any suitable method for isolating total RNA from a cell or tissue may be used. Typical procedures contemplated by the invention are described in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Ausubel, *et al.*, eds) (John Wiley & Sons, Inc. 1997), hereby incorporated by reference, at page 4.2.1 through page 4.2.7. Preferably, techniques which favor isolation of tRNA are employed as, for example, described in Brunngraber, E.F. (1962, *Biochem. Biophys. Res. Commun.* **8**:1-3) which is hereby incorporated by reference.

The probing of an RNA extract is suitably effected with different oligonucleotide sequences each being specific for a particular iso-tRNA. Of course it will be appreciated that for a given mammal, oligonucleotide sequences would need to be selected which hybridize specifically with particular iso-tRNA sequences expressed by the mammal. Such selection is well within the realm of one of ordinary skill in the art based a known iso-tRNA sequence. For example, in the case of a mouse, exemplary oligonucleotide sequences which may be used include those described in Gauss and Sprinzel (1983, *Nucleic Acids Res.* **11** (1)) hereby incorporated by reference. In this respect, the oligonucleotide sequences may be selected from the group consisting of:

5'-TAAGGACTGTAAGACTT-3' (SEQ ID NO:13) for Ala^{GCA}

5'-CGAGCCAGCCAGGAGTC-3' (SEQ ID NO:14) for Arg^{CGA}

5'-CTAGATTGGCAGGAATT-3' (SEQ ID NO:15) for Asn^{AAC}

5'-TAAGATATATAGATTAT-3' (SEQ ID NO:16) for Asp^{GAC}

5'-AAGTCTTAGTAGAGATT-3' (SEQ ID NO:17) for Cys^{TGC}

5'-TATTTCTACACAGCATT-3' (SEQ ID NO:18) for Glu^{GAA}

5'-CTAGGACAATAGGAATT-3' (SEQ ID NO:19) for Gln^{CAA}

5'-TACTCTCTTCTGGGTTT-3' (SEQ ID NO:20) for Gly^{GGA}

5'-TGCCGTGACTCGGATTC-3' (SEQ ID NO:21) for His^{CAC}

5'-TAGAAATAAGAGGGCTT-3' (SEQ ID NO:22) for Ile^{ATC}

5'-TACTTTTATTTGGATTT-3' (SEQ ID NO:23) for Leu^{CTA}

5'-TATTAGGGAGAGGATTT-3' (SEQ ID NO:24) for Leu^{CTT}

5'-TCACTATGGAGATTTTA-3' (SEQ ID NO:25) for Lys^{AAA}

5'-CGCCCAACGTGGGGCTC-3' (SEQ ID NO:26) for Lys^{AAG}

5'-TAGTACGGGAAGGATTT-3' (SEQ ID NO:27) for Met^{elong}

5'-TGTTTATGGGATACAAT-3' (SEQ ID NO:28) for Phe^{TTC}

5'-TCAAGAAGAAGGAGCTA-3' (SEQ ID NO:29) for Pro^{CCA}

5'-GGGCTCGTCCGGGATTT-3' (SEQ ID NO:30) for Pro^{CCI}

5'-ATAAGAAAGGAAGATCG-3' (SEQ ID NO:31) for Ser^{AGC}

5'-TGTCTTGAGAAGAGAAG-3' (SEQ ID NO:32) for Thr^{ACA}

5'-TGGTAAAAAGAGGATTT-3' (SEQ ID NO:33) for Tyr^{TAC}

5'-TCAGAGTGTTCATTGGT-3' (SEQ ID NO:34) for Val^{GTA}

Typically, the relative abundance of iso-tRNA species may be determined by blotting techniques that include a step whereby sample RNA or tRNA extract is immobilized on a matrix (preferably a synthetic membrane such as nitrocellulose), a hybridization step, and a detection step. Northern blotting may be used to identify an RNA sequence that is complementary to a nucleic acid probe. Alternatively, dot blotting

and slot blotting can be used to identify complementary DNA/RNA or RNA/RNA nucleic acid sequences. Such techniques are well known by those skilled in the art, and have been described in Ausubel, *et al* (*supra*) at pages 2.9.1 through 2.9.20.

According to such methods, a sample of tRNA immobilized on a matrix is hybridized under stringent conditions to a complementary nucleotide sequence (such as those mentioned above) which is labeled, for example, radioactively, enzymatically or fluorochromatically.

"Stringency" as used herein, refers to the temperature and ionic strength conditions, and presence or absence of certain organic solvents, during hybridization. The higher the stringency, the higher will be the degree of complementarity between the immobilized nucleotide sequences (*i.e.*, iso-tRNA) and the labeled oligonucleotide sequence. For a discussion of typical stringent conditions that may be used, see CURRENT PROTOCOLS IN MOLECULAR BIOLOGY *supra* at pages 2.10.1 to 2.10.16, and Sambrook *et al* in MOLECULAR CLONING. A LABORATORY MANUAL (Cold Spring Harbor Press, 1989), hereby incorporated by reference, at sections 1.101 to 1.104.

While stringent washes are typically carried out at temperatures from about 42°C to 68°C, one skilled in the art will appreciate that other temperatures may be suitable for stringent conditions. Maximum hybridization typically occurs at about 20° to 25° below the T_m for formation of a DNA-DNA hybrid. It is well known in the art that the T_m is the melting temperature, or temperature at which two complementary

nucleic acid sequences dissociate. Methods for estimating T_m are well known in the art (see CURRENT PROTOCOLS IN MOLECULAR BIOLOGY *supra* at page 2.10.8). Maximum hybridization typically occurs at about 10° to 15° below the T_m for a DNA-RNA hybrid.

Other stringent conditions are well known in the art. A skilled addressee will recognize that various factors can be manipulated to optimize the specificity of the hybridization. Optimization of the stringency of the final washes can serve to ensure a high degree of hybridization.

Methods for detecting labeled nucleotide sequences hybridized to an immobilized nucleotide sequence are well known to practitioners in the art. Such methods include autoradiography, chemiluminescent, fluorescent and colorimetric detection.

Advantageously, the relative abundance of an iso-tRNA in two or more cells or tissues may be determined by comparing the respective levels of binding of a labeled nucleotide sequence specific for the iso-tRNA to equivalent amounts of immobilized RNA obtained from the two or more cells or tissues. Similar comparisons are suitably carried out to determine the respective relative abundance of other iso-tRNAs in the two or more cells or tissues. One of ordinary skill in the art will thereby be able to determine a relative tRNA abundance table (see for example TABLE 2) for different cells or tissues. From such comparisons, one or more synonymous codons may be selected such that the or each synonymous codon corresponds to an iso-tRNA which, when compared to an

iso-tRNA corresponding to an existing codon of the parent nucleic acid sequence, is in higher abundance in the target cell or tissue relative to other cells or tissues of the mammal.

Advantageously, a synonymous codon is selected such that its corresponding iso-tRNA in the target cell or tissue is at a level which is at least 110%, preferably at least 200%, more preferably at least 500%, and most preferably at least 1000%, of that expressed in the or each other cell or tissue of the mammal.

Suitably, synonymous codons for selective expression of a protein in a differentiated cell, preferably a differentiated keratinocyte, are selected from the group consisting of gca (Ala), cuu (Leu) and cua (Leu).

Synonymous codons for selective expression of a protein in an undifferentiated cell, preferably an undifferentiated keratinocyte, are suitably selected from the group consisting of cga (Arg), cci (Pro) and aag (Asn).

Analysis of codon usage

Alternatively, synonymous codons may be selected by analyzing the frequency at which codons are used by genes expressed in (i) particular cells or tissues, (ii) substantially all cells or tissues of the mammal, or (iii) an organism which may infect particular cells or tissues of the mammal.

Codon frequency tables as well as suitable methods for determining frequency of codon usage in an organism are described, for example, in an article by

Sharp *et al* (1988, *Nucleic Acids Res.* 16 8207-8211) which is hereby incorporated by reference.

The relative level of gene expression (*e.g.*, detectable protein expression vs no detectable protein expression) can provide an indirect measure of the relative abundance of specific iso-tRNAs expressed in different cells or tissues. For example, a virus may be capable of propagating within a first cell or tissue (which may include a cell or tissue at a specific stage of differentiation) but may be substantially incapable of propagating in a second cell or tissue (which may include a cell or tissue at another stage of differentiation). Comparison of the pattern of codon usage by genes of the virus with the pattern of codon usage by genes expressed in the second cell or tissue may thus provide indirectly a set of synonymous codons which correspond to iso-tRNAs expressed at relatively high abundance in the first cell or tissue relative to the second cell or tissue and *vice versa*. Simultaneously, the above comparison may also provide indirectly a set of synonymous codons which correspond to iso-tRNAs expressed at relatively high abundance in the second cell or tissue relative to the first cell or tissue.

From the foregoing, a synonymous codon according to the invention may correspond to a codon including, but not limited to, (1) a codon used at relatively high frequency by genes, preferably highly expressed genes, of the target cell or tissue, (2) a codon used at relatively high frequency by genes, preferably highly expressed genes, of the or each other cell or tissue, (3) a codon used at relatively

high frequency by genes, preferably highly expressed genes, of the mammal, (4) a codon used at relatively low frequency by genes of the target cell or tissue, (5) a codon used at relatively low frequency by genes of the or each other cell or tissue, (6) a codon used at relatively low frequency by genes of the mammal, (7) a codon used at relatively high frequency by genes of another organism, and (8) a codon used at relatively low frequency by genes of another organism.

For example, codons used at a relatively high frequency by genes, preferably highly expressed genes, of the mammal may be selected from the group consisting of: cuc (Leu), cuu, (Leu), cug (Leu), uua (Leu), uug (Leu); cgg (Arg), cgc (Arg), aga (Arg), agg (Arg); agu (Ser), agc (Ser), ucu (Ser), ucc (Ser), and uca (Ser). Alternatively, such codons may include auu (Ile), auc (Ile); guu (Val), guc (Val), gug (Val); acu (Thr), acc (Thr), aca (Thr); gcu (Ala), gcc (Ala), gca (Ala); cag (Glu); ggc (Gly), gga (Gly), ggg (Gly).

Codons used at a relatively low frequency by genes of the mammal are described, for example, in Sharp *et al* (1988, *supra*). Such codons may comprise cua (Leu); cga (Arg), cgu (Arg); ucg (Ser). Alternatively, such codons may include aua (Ile); gua (Val); acg (Thr); gcg (Ala); caa (Glu); ggu (Gly).

Construction of synthetic nucleic acid sequences

The step of replacing synonymous codons for existing codons may be effected by any suitable technique. For example, *in vitro* mutagenesis methods may be employed which are well known to those of skill in the art. Suitable mutagenesis methods are

described for example in the relevant sections of Ausubel, *et al.* (*supra*) and of Sambrook, *et al.*, (*supra*) which are hereby incorporated by reference. Alternatively, suitable methods for altering DNA are set forth, for example, in U.S. Patent Nos 4,184,917, 4,321,365 and 4,351,901, which are hereby incorporated by reference. Instead of *in vitro* mutagenesis, the second nucleic acid sequence may be synthesized *de novo* using readily available machinery. Sequential synthesis of DNA is described, for example, in U.S. Patent No 4,293,652, which is hereby incorporated by reference. However, it should be noted that the present invention is not dependent on and not directed to any one particular technique for replacing synonymous codons for existing codons.

It is not necessary to replace all the existing codons of the parent nucleic acid sequence with synonymous codons each corresponding to a iso-tRNA expressed in relatively high abundance in the target cell compared to other cells. Increased expression may be accomplished even with partial replacement. Preferably, the replacing step affects 5%, 10%, 15%, 20%, 25%, 30%, more preferably 35%, 40%, 50%, 60%, 70% or more of the existing codons of the parent nucleic acid sequence.

The parent nucleic acid sequence is preferably a natural gene. By "natural gene" is meant a gene that naturally encodes the protein. However, it is possible that the parent nucleic acid sequence encodes a protein that is not naturally-occurring but has been engineered using recombinant techniques.

The parent nucleic acid sequence need not be obtained from the mammal but may be obtained from any suitable source such as from a eukaryotic or prokaryotic organism. For example, the parent nucleic acid sequence may be obtained from another mammal or other animal. Alternatively, the parent nucleic acid sequence may be obtained from a pathogenic organism. In such a case, a natural host of the pathogenic organism is preferably a mammal. For example, the pathogenic organism may be a yeast, bacterium or virus.

For example, suitable proteins which may be used for selective expression in accordance with the invention include, but are not limited to the cystic fibrosis transmembrane conductance regulator (CFTR) protein, and adenosine deaminase (ADA). In the case of CFTR, a parent nucleic acid sequence encoding the CFTR protein which may be utilized to produce the synthetic nucleic acid sequence is described, for example, in Riordan *et al* (1989, *Science* **245** 1066-1073), and in the GenBank database under Accession No. HUMCFTRM, which are hereby incorporated by reference.

The term "nucleic acid sequence" as used herein designates mRNA, RNA, cRNA, cDNA or DNA.

Regulatory nucleotide sequences which may be utilized to regulate expression of the synthetic nucleic acid sequence include, but are not limited to, a promoter, an enhancer, and a transcriptional terminator. Such regulatory sequences are well known to those of skill in the art.

Synthetic nucleic acid sequences according to the invention may be operably linked to one or more

regulatory sequences in the form of an expression vector. By "vector" is meant a nucleic acid molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, or mammalian or insect virus, into which a synthetic nucleic acid sequence may be inserted or cloned. A vector preferably contains one or more unique restriction sites and may be capable of autonomous replication in a defined host cell including the target cell or tissue or a precursor cell or precursor tissue thereof, or be integratable with the genome of the defined host such that the cloned sequence is reproducible. Thus, by "expression vector" is meant any autonomous element capable of directing the synthesis of a protein. Such expression vectors are well known by practitioners in the art.

The term "precursor cell" as used herein refers to a cell that gives rise to the target cell.

The invention also contemplates synthetic nucleic acid sub-sequences encoding desired portions of the protein. A nucleic acid sub-sequence encodes a domain of the protein having a function associated therewith and preferably encodes at least 10, 20, 50, 100, 150, or 500 contiguous amino acids of the protein.

The step of introducing the synthetic nucleic acid sequence into a target cell will differ depending on the intended use and or species, and may involve non-viral and viral vectors, cationic liposomes, retroviruses and adenoviruses such as, for example, described in Mulligan, R.C., (1993 *Science* **260** 926-932) which is hereby incorporated by reference. Such methods may include:

(i) Local application of the synthetic nucleic acid sequence by injection (Wolff *et al.*, 1990, *Science* **247** 1465-1468, which is hereby incorporated by reference), surgical implantation, instillation or any other means. This method may also be used in combination with local application by injection, surgical implantation, instillation or any other means, of cells responsive to the protein encoded by the synthetic nucleic acid sequence so as to increase the effectiveness of that treatment. This method may also be used in combination with local application by injection, surgical implantation, instillation or any other means, of another factor or factors required for the activity of said protein.

(ii) General systemic delivery by injection of DNA, (Calabretta *et al.*, 1993, *Cancer Treat. Rev.* **19** 169-179, which is hereby incorporated by reference), or RNA, alone or in combination with liposomes (Zhu *et al.*, 1993, *Science* **261** 209-212, which is hereby incorporated by reference), viral capsids or nanoparticles (Bertling *et al.*, 1991, *Biotech. Appl. Biochem.* **13** 390-405, which is hereby incorporated by reference) or any other mediator of delivery. Improved targeting might be achieved by linking the synthetic nucleic acid sequence to a targeting molecule (the so-called "magic bullet" approach employing for example, an antibody), or by local application by injection, surgical implantation or any other means, of another factor or factors required for the activity of the protein produced from said synthetic nucleic acid sequence, or of cells responsive to said protein.

(iii) Injection or implantation or delivery by any means, of cells that have been modified *ex vivo* by transfection (for example, in the presence of calcium phosphate: Chen *et al.*, 1987, *Mole. Cell Biochem.* **7** 2745-2752, or of cationic lipids and polyamines: Rose *et al.*, 1991, *BioTech.* **10** 520-525, which articles are hereby incorporated by reference), infection, injection, electroporation (Shigekawa *et al.*, 1988, *BioTech.* **6** 742-751, which is hereby incorporated by reference) or any other way so as to increase the expression of said synthetic nucleic acid sequence in those cells. The modification may be mediated by plasmid, bacteriophage, cosmid, viral (such as adenoviral or retroviral; Mulligan, 1993, *Science* **260** 926-932; Miller, 1992, *Nature* **357** 455-460; Salmons *et al.*, 1993, *Hum. Gen. Ther.* **4** 129-141, which articles are hereby incorporated by reference) or other vectors, or other agents of modification such as liposomes (Zhu *et al.*, 1993, *Science* **261** 209-212, which is hereby incorporated by reference), viral capsids or nanoparticles (Bertling *et al.*, 1991, *Biotech. Appl. Biochem.* **13** 390-405, which is hereby incorporated by reference), or any other mediator of modification. The use of cells as a delivery vehicle for genes or gene products has been described by Barr *et al.*, 1991, *Science* **254** 1507-1512 and by Dhawan *et al.*, 1991, *Science* **254** 1509-1512, which articles are hereby incorporated by reference. Treated cells may be delivered in combination with any nutrient, growth factor, matrix or other agent that will promote their survival in the treated subject.

In yet another aspect, the invention provides a pharmaceutical composition comprising the synthetic nucleic sequences of the invention and a pharmaceutically acceptable carrier.

By "pharmaceutically-acceptable carrier" is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in systemic administration. Depending upon the particular route of administration, a variety of pharmaceutically acceptable carriers, well known in the art may be used. These carriers may be selected from a group including sugars, starches, cellulose and its derivatives, malt, gelatin, talc, calcium sulfate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline, and pyrogen-free water.

Any suitable technique may be employed for determining expression of the protein from said synthetic nucleic acid sequence in a particular cell or tissue. For example, expression can be measured using an antibody specific for the protein of interest or portion thereof. Such antibodies and measurement techniques are well known to those skilled in the art.

Applications

In one embodiment of the present invention, the target cell is suitably a differentiated cell. Advantageously, the protein which is desired to be selectively expressed in the differentiated cell is not expressible in a precursor cell thereof (such as an undifferentiated or less differentiated cell of the mammal) from a parent nucleic acid sequence at a level

sufficient to effect a particular function associated with said protein. In this embodiment, the step of replacing at least one existing codon with a synonymous codon is characterized in that the synonymous codon corresponds to an iso-tRNA which, when compared to the iso-tRNA corresponding to the at least one existing codon, is in relatively higher abundance in the differentiated cell compared to the precursor cell. Accordingly, a synthetic nucleic acid sequence is produced having altered translational kinetics compared to the parent nucleic acid sequence wherein the protein is expressible in the differentiated cell at a level sufficient to effect a particular function associated with said protein, but wherein the protein is not expressible in the precursor cell at a level sufficient to effect said function.

As used herein, the term "function" refers to a biological, or therapeutic function.

The above embodiment may be utilized advantageously for somatic gene therapy where overexpression of a protein in undifferentiated cells such as stems cells has undesirable consequences including death or differentiation of the stem cells. In such a case, a suitable protein may include cystic fibrosis transmembrane conductance regulator (CFTR) protein, and adenosine deaminase (ADA).

The differentiated cell may comprise a cell of any lineage including a cell of epithelial, hemopoetic or neural origin. For example, the differentiated cell may be a mature differentiated keratinocyte.

Targeting expression of a protein to progeny of a stem cell but not to the stem cell itself

The synthetic nucleic acid sequence produced above may be transfected directly into the differentiated cell for the desired function or alternatively, transfected into the precursor cell. For example, in the case of ADA deficiency, expression of ADA in stem cells may result in loss of stem phenotype which is undesirable. However, an advantageous therapy may reside in transducing autologous marrow stem cells with a synthetic nucleic acid sequence operably linked to one or more regulatory sequences, wherein existing codons of the wild type ADA gene have been replaced with synonymous codons each corresponding to an iso-tRNA expressed in relatively high abundance in differentiated lymphocytes compared to the marrow stem cells. The transduced stem cells may then be reinfused into the patient. This approach will result in transduced marrow stem cells which are not capable of expressing ADA themselves, but which are able to give rise to a renewable population of differentiated lymphocytes which are capable of expressing ADA at levels sufficient to permit a therapeutic effect. In this regard, a suitable cell source for this purpose may comprise stem cells isolated as CD34 positive cells from a patient's peripheral blood or marrow. For gene delivery, a suitable vector may include a retrovirus or Adeno associated virus.

Alternatively, in the case of inducing cell mediated immunity, dendritic cells are important

antigen presenting cells (APC) but have a very limited life span for antigen presentation once activated of between 14 to 21 days. Consequently, dendritic cells provide relatively short-term immune stimulation that may not be optimal. However, in accordance with the present invention, a long-term immune stimulation may be provided by transducing autologous bone marrow-derived CD34 positive dendritic cell precursors with a synthetic nucleotide sequence encoding an antigen. such as the melanoma antigen MART-1, wherein the synthetic sequence is operably linked to one or more regulatory sequences, and wherein existing codons of a wild type nucleotide sequence encoding MART-1 have been replaced with synonymous codons each corresponding to an iso-tRNA expressed in relatively high abundance in dendritic cells compared to the dendritic cell precursors. The transduced dendritic cell precursors may then be reinfused into the patient. This approach will result in transduced dendritic cell precursors which are not capable of expressing MART-1 themselves, but which are able to give rise to a renewable population of dendritic cells which are capable of expressing MART-1 at levels sufficient to permit a lifelong intermittent restimulation of a cytotoxic T lymphocyte (CTL) response to the MART-1 antigen.

Targeting expression of a protein to a stem cell but not to progeny of the stem cell

In an alternate embodiment, the target cell may be an undifferentiated cell wherein the protein is not expressible in said undifferentiated cell, from a

parent nucleic acid sequence encoding the protein, at a level sufficient to effect a particular function associated with the protein. In such a case, at least one existing codon of the parent nucleic acid sequence is replaced with a synonymous codon corresponding to an iso-tRNA which, when compared to the iso-tRNA corresponding to the at least one existing codon, is in relatively higher abundance in the undifferentiated cell compared to a differentiated cell. This results in a synthetic nucleic acid sequence having altered translational kinetics compared to said parent nucleic acid sequence wherein the protein is expressible in the undifferentiated cell at a level sufficient to effect a particular function associated with the protein, but wherein the protein is not expressible in differentiated cells derived from the undifferentiated cell at a level sufficient to effect said function.

This alternate embodiment may, by way of example, be used to permit expression of a transcriptional regulatory protein which when expressed in a particular undifferentiated cell or stem cell facilitates differentiation of the stem cell along a particular cell lineage. It will be appreciated that in such a case, the regulatory protein is normally expressed from a gene in which the existing codons correspond to iso-tRNAs which are in relatively low abundance in the stem cell compared to other iso-tRNAs and that therefore the protein is not capable of being expressed at levels sufficient for commitment of the stem cell to differentiate along a particular cell lineage. It will also be apparent that such commitment to differentiate along a

particular cell lineage may be utilized to prevent production of a particular lineage of cells such as cancer cells.

Alternatively, the method according to this embodiment may be used to express a transcriptional regulatory protein that is involved in the production of a therapeutic agent or agents. Such a protein may include, for example, NF-kappa-B transcription factor p65 subunit (NF-kappa-B p65) which is involved in the production of interleukin-2 (IL-2), interleukin-3 (IL-3) and granulocyte and macrophage colony stimulating factor (GMCSF). NF-kappa-B p65 is encoded naturally by a nucleotide sequence comprising a number of existing codons each corresponding to an iso-tRNA expressed in relatively low abundance in stem cells. Accordingly, such sequence may be used as the parent nucleic acid sequence according to this embodiment. A suitable nucleotide sequence encoding this protein is described, for example, in Lyle *et al* (1994, *Gene* **138** 265-266) and in the EMBL database under Accession No HSNFKB65A which are hereby incorporated by reference.

A suitable undifferentiated cell which may be utilized in accordance with the present embodiment includes but is not limited to a stem cell, such as a CD34 positive hemopoetic stem cell.

The present embodiment may also be used advantageously for gene therapy where ongoing regulated expression of a transgene is desirable. For example, secure but reversible regulation of fertility is desirable in veterinary practice and in humans. Such regulation may be effected by transducing autologous breast ductal epithelial cells with a

synthetic nucleic acid encoding a leutinising hormone (LH) antagonist or a leutinising hormone releasing hormone (LHRH) antagonist under the control of one or more regulatory sequences. The synthetic nucleic acid may be produced by replacing existing codons of a parent nucleic acid with synonymous codons corresponding to iso-tRNAs expressed in relatively high abundance in resting breast ductal epithelial cells compared to differentiated cells arising therefrom. Once the transduced cells are implanted back into the patient, expression may be switched off by oral administration of progestagen, forcing the differentiation of the majority of the stem cells and loss of expression of the antagonist. Once pregnancy is established, the suppression would be self sustaining by the naturally produced progestagen. The iso-tRNA composition of resting and oestrogen drived breast epithelial cells may be established by first obtaining resting cells from reduction mammoplasty, and determining the cellular tRNA composition in the presence and absence of oestrogen. The synthetic nucleic acid sequence may be introduced into autologous resting epithelial cells by cell electroporation *ex vivo*, and the transduced cells may be subsequently transplanted subcutaneously into the patient. Progestagen may be administered as required to reverse regulation of fertility.

Targeting expression of a toxin to a tumor cell but not to any other cells of the mammal

Many toxins and drugs are available that can kill tumor cells. However, these toxins and drugs

are generally toxic for all dividing cells. This problem may be nevertheless ameliorated by establishing the isoacceptor tRNA composition in a tumor clone, and constructing a synthetic toxin gene (*e.g.*, ricin gene) or a synthetic anti-proliferation gene (*e.g.*, the tumor supressor p53) using synonymous codons corresponding to iso-tRNAs expressed at relatively high abundance in the tumor clone compared to normal dividing cells of the mammal. The synthetic gene is then introduced into the patient by suitable means to selectively express the synthetic genes in tumor cells.

Alternatively, a chemotherapy enhancing product gene (*i.e.*, a drug resistance gene *e.g.*, the multi-drug resistance gene) using a codon pattern unlikely to be expressed in the tumor efficiently may be employed.

Targeting gene therapy to control body fat

Leptins are proteins known to control satiety. By analogy with animal data, however, if too much leptin is administered to a patient, leptin-induced starvation might occur. Advantageously, a synthetic gene encoding leptin may be constructed including synonymous codons corresponding to iso-tRNAs expressed at relatively high levels in activated adipocytes compared to non-activated adipocytes. The synthetic gene may then be introduced into the patient by suitable means such that leptin is only expressed substantially in activated adipocytes as opposed to non-activated adipocytes. As body fat turnover diminishes under the influence of leptin reduced

appetite, the metabolic activity of the adipocytes falls and the leptin production decreases correspondingly.

Targeting expression of a protein to a stage of the cell cycle

In another embodiment of the invention, the target cell may be a non-cycling cell. In this case, the protein which is desired to be selectively expressed in the non-cycling cell is expressible in a cycling cell of the mammal from a parent nucleic acid sequence at a level sufficient to effect a particular function associated with the protein. The synonymous codons are selected such that each corresponds to an iso-tRNA which, when compared to the iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the non-cycling cell compared to the cycling cell. Accordingly, a synthetic nucleic acid sequence is produced having altered translational kinetics compared to the parent nucleic acid sequence wherein the protein is expressible in the non-cycling cell at a level sufficient to effect a particular function associated with said protein, but wherein the protein is not expressible in the non-cycling cell to effect said function.

The term "non-cycling cell" as used herein refers to a cell that has withdrawn from the cell cycle and has entered the G0 state. In this state, it is well known that transcription of endogenous genes and protein translation are at substantially reduced levels compared to phases of the cell cycle, namely G1, S, G2 and M.

By "cycling cell" is meant a cell which is in one of the above phases of the cell cycle.

Expressing a protein in a target cell or tissue by in vivo expression of iso-tRNAs in the target cell or tissue

In another aspect, the invention extends to a method wherein a protein may be selectively expressed in a target cell by introducing into the cell an auxiliary nucleic acid sequence capable of expressing therein one or more isoaccepting transfer RNAs which are not expressed in relatively high abundance in the cell but which are rate limiting for expression of the protein from a parent nucleic acid sequence to a level sufficient for effecting a function associated with the protein. In this embodiment, introduction of the auxiliary nucleic acid sequence in the cell changes the translational kinetics of the parent nucleic acid sequence such that said protein is expressed at a level sufficient to effect a function associated with the protein.

The step of introducing the auxiliary nucleic acid sequence into the target cell or a tissue comprising a plurality of these cells may be effected by any suitable means. For example, analogous methodologies for introduction of the synthetic nucleic acid sequence referred to above may be employed for delivery of the auxiliary nucleic acid sequence into said cycling cell.

Assembly of virus particles in cells which do not normally permit assembly of virus particles

In yet another aspect, the invention extends to a method for producing a virus particle in a cycling eukaryotic cell. The virus particle will comprise at least one protein necessary for virus assembly, wherein the at least one protein is not expressed in the cell from a parent nucleic acid sequence at a level sufficient to permit virus assembly therein. This method is characterized by replacing at least one existing codon of the parent nucleic acid sequence with a synonymous codon to produce a synthetic nucleic acid sequence having altered translational kinetics compared to the parent nucleic acid sequence such that the at least one protein is expressible from the synthetic nucleic acid sequence in the cell at a level sufficient to permit virus assembly therein. The synthetic nucleic acid sequence so produced is operably linked to one or more regulatory nucleotide sequences and is then introduced into the cell or a precursor cell thereof. The at least one protein is expressed subsequently in the cell in the presence of other viral proteins required for assembly of the virus particle to thereby produce the virus particle.

Advantageously, the synonymous codon corresponds to an iso-tRNA expressed at relatively high level in the cell compared to the iso-tRNAs corresponding to the existing codons.

The cycling cell may be any cell in which the virus is capable of replication. Suitably, the cycling cell is a eukaryotic cell. Preferably, the

cycling cell for production of the virus particle is a eukaryotic cell line capable of being grown *in vitro* such as, for example, CV-1 cells, COS cells, yeast or spodoptera cells.

Suitably, the at least one protein of the virus particle are viral capsid proteins. Preferably, the viral capsid proteins comprise L1 and/or L2 proteins of papillomavirus.

The other viral proteins required for assembly of the virus particle in the cell may be expressed from another nucleic acid sequence(s) which suitably contain the rest of the viral genome. In the case of the at least one protein comprising L1 and/or L2 of papillomavirus, said other nucleic acid sequence(s) preferably comprises the papillomavirus genome without the nucleotide sequences encoding L1 and/or L2.

In yet a further aspect of the invention, there is provided a method for producing a virus particle in a cycling cell, said virus particle comprising at least one protein necessary for assembly of said virus particle, wherein said at least one protein is not expressed in said cell from a parent nucleic acid sequence at a level sufficient to permit virus assembly therein, and wherein at least one existing codon of said parent nucleic acid sequence is rate limiting for the production said at least one protein to said level, said method including the step of introducing into said cell a nucleic acid sequence capable of expressing therein an isoaccepting transfer RNA specific for said at least one codon.

In yet a further aspect, the invention resides in virus particles resulting from the above methods.

The invention further contemplates cells or tissues containing therein the synthetic nucleic acid sequences of the invention, or alternatively, cells or tissues produced from the methods of the invention.

The invention is further described with reference to the following non-limiting examples.

EXAMPLE 1

Expression of synthetic L1 and L2 protein in undifferentiated cells.

Materials and Methods

Codon replacements in the bovine PV (BPV) L1 and L2 genes

The DNA and amino acid sequences of the wild-type L1 (SEQ ID NOS:1,2)and L2 genes (SEQ ID NOS:5,6) are shown respectively in Figures 1A and 1B. To determine whether the presence of rare codons in wild-type L1 (SEQ ID NO:1) and L2 (SEQ ID NO:5) genes (Table 1) inhibited translation, we synthesized the L1 (SEQ ID NO:3) and L2 (SEQ ID NO:7) genes by using synonymous substitutions as shown. To construct the synthetic sequences, we synthesized 11 pairs of oligonucleotides for L1 and 10 pairs of oligonucleotides for L2. Each pair of oligonucleotides has restriction sites incorporated to facilitate subsequent cloning (Figures 1A and 1B). The degenerate oligonucleotides were used to amplify

L1 and L2 sequences by PCR using a plasmid with BPV1 genome as the template. The amplified fragments were cut with appropriate enzymes and sequentially ligated to pUC18 vector, producing pUCHBL1 and pUCHBL2. The synthetic L1 (SEQ ID NO:3) and L2 (SEQ ID NO:7) sequences were sequenced and found to be error-free, and then sub-cloned into the mammalian expression vector pCDNA3 containing SV40 *ori* (Invitrogen), giving expression plasmids pCDNA/HBL1 and pCDNA/HBL2. To compare expression of L1 and L2 with that of the original sequences, the wild type L1 (SEQ ID NO:1) and L2 (SEQ ID NO:5) genes were cloned into the pCDNA3 vector, resulting in pCDNA/BPVL1wt and pCDNA/BPVL2wt.

Immunofluorescence and Western blot staining

For immunoblotting assays, Cos-1 cells in 6-well plates were transfected with 2 μg L1 or L2 expression plasmids using lipofectamine (Gibco). 36 hrs after transfection, cells were washed with 0.15M phosphate buffered 0.9% NaCl (PBS) and lysed in SDS loading buffer. The cellular proteins were separated by 10% SDS PAGE and blotted onto nitrocellulose membrane. The L1 or L2 proteins were identified by electrochemiluminescence (Amersham, UK), using BPV1 L1 (DAKO) or L2-specific (17) antisera. For immunofluorescent staining, Cos-1 cells were grown on 8-chamber slides, transfected with plasmids, and fixed and permeabilised with 85% ethanol 36hr after transfection. The slides were blocked with 5% milk-PBS and probed with L1 or L2-specific antisera, followed by FITC-conjugated anti-rabbit IgG (Sigma). For GFP or

PGFP plasmid transfected cells, the cell were fixed with 4% buffered formaldehyde and viewed by epi-fluorescence microscopy.

Northern blotting

Cos cells transfected with various plasmids were used to extract cytoplasmic or total RNA using the QIAGEN RNeasy mini kit according to the supplier's handbook. Briefly, for cytoplasmic RNA purification, buffer RLN (50 mM Tris, pH 8.0, 140 mM NaCl, 1.5 mM $MgCl_2$ and 0.5% NP40) was directly added to monolayer cells and cells were lysed in 4 °C for 5 min. After the nuclei were removed by centrifugation, cytoplasmic RNAs were purified by column. For total RNA extraction, the monolayer cells were lysed using buffer RLT supplied by the kit and RNA was purified by spin column. The purified RNAs were separated by 1.5% agarose gel in the presence of formaldehyde. The RNAs were then blotted onto nylon membrane and probed with (a) 1:1 mixed 5'-end labelled L1 wt and HBL1 fragments; (b) 1:1 mixed 5'-end labelled L2 wt and HBL2 fragments; (c) 1:1 mixed 5'end labelled GFP and PGFP fragments or (d) randomly labelled PAGDH fragment. The blots were washed extensively at 65 °C and exposed to X-ray films for three days.

Results

To test the hypothesis that the codon composition of the genes encoding the L1 and L2 capsid proteins of papillomavirus (PV) contributes to their preferential expression in differentiated epithelial

cells, we produced synthetic BPV1 L1 (SEQ ID NO:3) and L2 (SEQ ID NO:7) genes, substituting codons preferentially used in mammalian genes for the codons frequently present in the wild type BPV1 L1 and L2 sequences which are rare in eukaryotic genes (Figures 1A, 1B).

For the L1 gene, a total of 202 base substitutions were made in 196 codons, without changing the encoded amino acid sequence (Figure 1A). This synthetic "humanized" BPV L1 gene (SEQ ID NO:3) was designated HBL1. In a similarly modified BPV1 L2 gene (SEQ ID NO:7) designated HBL2, 303 bases were changed to substitute 290 less frequently used codons with the corresponding preferentially used codons. Using the synthetic HBL1 (SEQ ID NO:3) and HBL2 (SEQ ID NO:7) genes, we constructed two eukaryotic expression plasmids based on pCDNA3, and designated pCDNA/HBL1 and pCDNA/HBL2. Similar expression plasmids, constructed with the wild type BPV1 L1 (SEQ ID NO:1) and BPV1 L2 (SEQ ID NO:5) genes, were designated pCDNA/BPVL1wt and pCDNA/BPVL2wt, respectively. In each of these plasmids the SV40 ori allowed replication in Cos-1 cells, and the L1 or L2 gene was driven by a strong constitutive CMV promoter.

To compare the expression of the synthetic humanized and the wild type BPV1 L1 or BPV1 L2 genes, we separately transfected Cos-1 cells with each of the L1 and L2 plasmids described above. Transfected cells were analyzed for expression of L1 (SEQ ID NO:2,4) or L2 (SEQ ID NO:6,8) protein by immunofluorescence 36 hr after transfection (Figures 2A and 3A). Cells transfected with the pCDNA3 expression plasmid

containing the synthetic humanized L1 (SEQ ID NO:3) or L2 (SEQ ID NO:7) genes were observed to produce large amounts of the corresponding protein, while cells transfected with expression plasmids with the wild type L1 (SEQ ID NO:1) or L2 (SEQ ID NO:5) sequences produced no detectable L1 or L2 protein (Figures 2A and 3A, see nuclear staining of L1 and L2 proteins). To compare more accurately the expression of the different L1 and L2 constructs, L1 and L2 protein expression was assessed by immunoblot in Cos-1 cells transfected with the wild type or synthetic humanized BPV1 L1 or L2 pCDNA3 expression constructs (Figures 2B and 3B). Large amounts of immunoreactive L1 and L2 proteins were expressed from the synthetic humanized L1 (SEQ ID NO:3) and L2 (SEQ ID NO:7) sequences, but no L1 or L2 protein was expressed from the wild type L1 and L2 sequences (SEQ ID NO:1,5).

To establish whether the alterations to the primary sequence of the L1 and L2 mRNA which resulted from the codon alterations also affected steady state expression of the corresponding message, mRNA was prepared from Cos-1 cells transfected with the various capsid protein gene constructs. Using GAPDH as an internal standard it was established by Northern blot that two to three times more modified than wild type L1 mRNA, and similar levels of wild type and modified L2 mRNA were present in the cytoplasm of transfected cells (Figures 2C and 3C). The amount of L1 or L2 protein expressed per arbitrary unit of L1 or L2 mRNA was at least 100 fold higher for the humanized gene constructs than for the natural gene constructs.

EXAMPLE 2

Papillomavirus late protein translation *in vitro*

Materials and Methods

In vitro translation assay

One microgram of each plasmid was incubated with 20 μCi ^{35}S-methionine (Amersham) and 40 μL T7 coupled rabbit reticulocyte or wheat germ lysates (Promega). Translation was performed at 30 °C and stopped by adding SDS loading buffer. The L1 proteins were separated by 10% SDS PAGE and examined by autoradiography.

Production of aminoacyl-tRNA

2.5 x 10^{-4} M tRNA (Boehringer) was added to a 20 μL reaction containing 10 mM Tris-acetate, pH.7.8, 44 mM KCl, 12 mM $MgCl_2$, 9 mM -mercaptoethanol, 38 mM ATP, 0.25 mM GTP and 7 μL rabbit reticulocyte extract. The reaction was carried out at 25 °C for 20 min, and 30 μL H_2O was added to the reaction to dilute the tRNAs to 1 x 10^{-4} M. The aminoacyl-tRNAs were then aliquoted and stored at -70 °C.

Results

As the major limitation to expression of the wild type BPV L1 and L2 genes appeared to be translational in our system we wished to test whether this limitation reflected a limited availability of the appropriate tRNA species for gene translation. As transient expression of the synthetic genes within

intact cells may be regulated by many factors, we tested our hypothesis in a cell free system using rabbit reticulocyte lysate (RRL) or wheat germ lysate to examine gene translation. Similar amounts of plasmids expressing the wild type or synthetic humanized BPV1 L1 gene were added to a T7-DNA polymerase coupled RRL transcription/translation system in the presence of ^{35}S-methionine. After 20 minutes, translated proteins were separated by SDS PAGE and visualized by autoradiography. Efficient translation of the modified L1 gene was observed (Figure 4, top panel, lane 2), while translation of the wild type BPV1 L1 sequence resulted in a weak 55 kDa L1 band (Figure 4, upper panel, lane 1). We reasoned that although the wild type sequence was not optimized for translation in RRL, some translation would occur as there would be no cellular mRNA species competing for the 'rare' codons present in the wild type L1 sequence. The above data suggest that the observed difference in efficiency of translation of the wild type and synthetic humanized L1 genes is a consequence of limited availability of the tRNAs required for translation of the rare codons present in the wild type gene. We therefore expected that addition of excess tRNA to the in vitro translation system would overcome the inhibition of translation of the wild type L1 gene. To address this question, 10^{-5} M aminoacyl-tRNAs from yeast were added into the RRL translation system, and L1 protein synthesis was assessed. Introduction of exogenous tRNAs resulted in a dramatic improvement in translation of the wild type L1 sequence, which now gave a yield of L1 protein

comparable to that observed with the synthetic humanized L1 sequence (SEQ ID NO:3) (Figure 4, top panel). Enhancement of translation of the wild type L1 gene (SEQ ID NO:1) by aminoacyl-tRNA was dose-dependent, with an optimum efficiency at 10^{-5} M tRNA. As addition of exogenous tRNA improved the yield of L1 protein translated from the wild type L1 gene sequence (SEQ ID NO:1), we assessed the speed of translation of wild type and humanized L1 mRNA. Samples were collected from the translation mixture every 2 minutes, starting at the 8th minute. Translation of L1 (SEQ ID NO:2,4) from the wild type sequence (SEQ ID NO:1) was much slower than from the humanized L1 sequence (SEQ ID NO:3) (Figure 4 bottom panel), and the retardation of translation could be completely overcome by adding exogenous tRNA from commercially available yeast tRNA. Yeast tRNA was chosen in the above analysis because the codon usage in yeast is similar to that of papillomavirus (Table 1). Addition of exogenous tRNA did not significantly improve the translation of the humanized L1 gene (SEQ ID NO:3), indicating that this sequence was optimized with regard to codon usage for the rabbit reticulocyte translation machinery (Figure 4, bottom panel). In separate experiments we established that wt L1 translation could also be enhanced by liver tRNA (Figure 4), and by tRNAs extracted from bovine skin epidermis, which presumably constitutes a mixture of tRNAs from differentiated and undifferentiated cells (data not shown).

EXAMPLE 3

Translation of wild type L1 is efficient in wheat germ extract.

To further test our hypothesis that tRNA availability is a determinant of expression of the wild type BPV1 L1 gene (SEQ ID NO:1), we examined the translation of L1 in a cell type in which a quite different set of tRNAs would be available. In a wheat germ translation system, wild type L1 mRNA was translated as efficiently as humanized L1 mRNA, and addition of exogenous aminoacyl-tRNAs did not improve the translation efficiency of either wild type or humanized sequences (Figure 4 bottom panel). This indicated that in wheat germ there are sufficient of the tRNAs which are limiting for translation of wild type L1 sequence in RRL to allow efficient L1 translation.

EXAMPLE 4

Modified late genes can be expressed in undifferentiated cells from papillomavirus promoter(s)

While our data presented above indicates that translation is limiting for the production of BPV1 capsid proteins in our test system, these experiments were conducted in systems which are not truly representative of the viral late gene transcription from the BPV genome, in part because the genes were driven by a strong CMV promoter. We therefore wished to establish whether synthetic

humanized BPV capsid protein mRNA would be translated more efficiently than the wild type mRNA, if transcribed from the natural BPV1 promoter. This would establish whether translation was indeed one of the limiting factors for expression of BPV1 late genes driven from the natural cryptic late gene promoter in an undifferentiated cell. The BPV genome was cleaved at nt 4450 and 6958 with *Bam*HI/*Hin*dIII and the original L1 (nt 4186-5595) and L2 (5068-7095) ORFs were removed. The synthetic humanized L2 gene (SEQ ID NO:7), together with an SV40 *ori* sequence to allow plasmid replication in eukaryotic cells, were inserted into the BPV genome lacking L1/L2 ORF sequences. This plasmid (Figure 5A) was designated pCICR1. A similar plasmid was constructed with wild type (SEQ ID NO:5) rather than synthetic humanized L2 and designated pCICR2. Cos-1 cells were transfected with these plasmids and L2 protein expression examined by immunofluorescence of transfected cells. Synthetic humanized L2 (SEQ ID NO:7), driven by the natural BPV-1 promoter, was efficiently expressed, whereas the wild type L2 sequence (SEQ ID NO:5), driven from a similar construct, produced no immunoreactive L2 protein (SEQ ID NO:6,8) (Figure 5B). As undifferentiated cells supported the expression of the humanized L2 gene (SEQ ID NO:7) but not the wild type L2 (SEQ ID NO:5) expressed from the cryptic late BPV promoter, the results confirmed our earlier observations from experiments using the CMV promoter. However, the plasmids tested here contained SV40 *ori*, designed to replicate the DNA in Cos cells. The increased copy number of the BPV1 L2 plasmids or the

transcriptional enhancing activity of the SV40 *ori* might explain in part the increased efficiency of expression of L2 in this experimental system when compared with infected skin. However, the marked difference in expression between the natural and humanized genes seen with a CMV promoter construct is still observed with the natural promoter.

EXAMPLE 5

Substitution of papillomavirus-preferred codons prevents translation but not transcription of a non-papillomavirus gene in undifferentiated cells.

Materials and Methods

Codon replacement in gfp gene

To construct a modified gfp gene (SEQ ID NO:11) using papillomavirus preferred codons (PGFP), 6 pairs of oligonucleotides were synthesized. Each pair of oligonucleotides has restriction sites incorporated and was used to amplify *gfp* using a humanized *gfp* gene (SEQ ID NO:9) (GIBCO) as template. The PCR fragments were ligated into the pUC18 vector to produce pUCPGFP. The PGFP gene was sequenced, and cloned into *Bam*HI site of the same mammalian expression vector, pCDNA3, under the CMV promoter. The DNA and deduced amino acid sequences of the humanized GFP gene are shown in Figures 1C. Mutations introduced into the wild type *gfp* gene (SEQ ID NO:9) to produce the P*gfp* gene (SEQ ID NO:11) are indicated above the corresponding nucleotides of the wild-type sequence.

Results

To further confirm that codon usage can alter gene expression in mammalian cells, we made a further variant on a synthetic *gfp* gene modified for optimal expression in eukaryotic cells (Zolotukhin, *et al.*, 1996. *J. Virol.* 70:4646-4654). In our variant, codons optimized for expression in eukaryotic cells were substituted by those preferentially used in papillomavirus late genes. Of 240 codons in the humanized *gfp* gene (SEQ ID NO:9), which expresses high levels of fluorescent protein in cultured cells, 156 were changed to the corresponding papillomavirus late gene-preferred codons to produce a new *gfp* gene (SEQ ID NO:11) designated P*gfp*. Expression of P*gfp* (SEQ ID NO:11) in undifferentiated cells was compared with that of humanized *gfp* (SEQ ID NO:9). Cos-1 cells transfected with the humanized *gfp* (SEQ ID NO:9) produced a bright fluorescent signal after 24 hrs, while cells transfected with P*gfp* (SEQ ID NO:11) produced only a faint fluorescent signal (Figure 6A). To confirm that this difference reflected differing translational efficacy, *gfp* specific mRNA was tested in both transfections and found not to be significantly different (Figure 6B.). Thus, codon usage and corresponding tRNA availability apparently determines the observed restriction of expression of PV late genes, and modification of codon usage in other genes similarly prevents their expression in undifferentiated cells.

EXAMPLE 6

PGFP with papillomavirus-preferred codons is efficiently expressed *in vivo* in differentiated mouse keratinocytes.

Materials and Methods

Delivery of plasmid DNA into mouse skin by gene gun

Fifty microgram of DNA was coated onto 25 µg gold micro-carriers by calcium precipitation, following the manufacturer's instructions (Bio-Rad). C57/bl mouse skin was bombarded with gold particles coated with DNA plasmid at a pressure of 600 psi. Serial sections were taken from the skin and examined for distribution of the particles, confirming that a pressure of 600 psi could deliver particles throughout the epidermis.

Results

Mice were shot with gold beads carrying PGFP DNA plasmid and, 24 hrs later, skin samples were cut from the site of DNA delivery and examined for expression of GFP protein (SEQ ID NO:10,12). Fluorescence was detected mostly in upper keratinocyte layers, representing the differentiated epithelium, and was not seen in undifferentiated basal cells. In contrast, skin sections shot with the humanized GFP plasmid showed fluorescence in cells randomly distributed throughout the whole epidermis (Figure 7). Although GFP-positive cells were rare in both PGFP- (SEQ ID NO:11) and GFP-inoculated (SEQ ID NO:9) mouse

skin, fluorescence was observed only in differentiated strata in the PGFP sample (SEQ ID NO:11), whereas fluorescence was observed throughout the epidermis in GFP-inoculated (SEQ ID NO:9) mouse skin. This result confirmed that the use of papillomavirus-preferred codons resulted in the protein being expressed in an epithelial differentiation-dependent manner.

EXAMPLE 7

Microinjection of yeast tRNA and wild type L1 gene into cultured cells

To test if yeast tRNA could facilitate expression of wild type BPV-1 L1 (SEQ ID NO:1) (as yeast uses a similar set of codons to those observed in papillomavirus for its own genes), 2 pL of mixtures containing tRNA (2 mg/mL) (purified yeast tRNA (Boehringer Mannheim) or bovine liver tRNA - control) and BPV L1 DNA (2 μg/mL) can be injected into CV-1 cells (Lu and Campisi, 1992, *Proc. Natl. Acad. Sci. U. S. A.* **89** 3889-3893). The injected cells can then be cultured for 48 hrs at 37 °C and examined for expression of L1 gene by standard immunofluoresence methods using BPV L1-specific antibody and quantified by FACS analysis (Qi *et al* 1996, *Virology* **216** 35-45).

EXAMPLE 8

Establishment of a cell line which can continuously produce HPV virus particles

To produce infectious PV, various methods have been tried including the epithelial raft culture

system (Dollard *et al* 1992, *Genes Dev* **6** 1131-1142), and cell lines containing BPV-1 episomal DNA, and infected by BPV-1 L1/L2 recombinant vaccinia (Zhou *et al* 1993, *J. Gen. Virol.* **74** 763-768) or transfected by SFV RNA (Roden *et al* 1996, *J. Virol.* **70** 5875-5883). The yield of particles is in each case low. In a reduction to practice of our discovery, synthetic BPV L1 (SEQ ID NO:3) and L2 genes (SEQ ID NO:7) (as described in Example 1) can be used to produce infectious BPV in a cell line containing BPV-1 episomal DNA. Fibroblast cell lines (CON/BPV) containing BPV-1 episomal DNA (Zhou *et al* 1993, *J. Gen. Virol.* **74** 763-768) can be used for transfection of the synthetic BPV-1 L1 (SEQ ID NO:3) and L2 genes (SEQ ID NO:7) under control of CMV promoter. BPV particles may then be purified from the cell lysate and the purified particles examined for the presence of BPV-1 genome. Standard methods such as transfection with lipofectamine (BRL) and G418 selection of transfected cells can be utilized to generate suitable transfectants expressing humanized L1 (SEQ ID NO:3) and L2 (SEQ ID NO:7) in the background of BPV-1 episomal DNA. Examination of L1 and L2 protein expression can be performed using rabbit anti-BPV L1 or rabbit anti-BPV L2 polyclonal antibodies. BPV particles can then be purified using our published methods (Zhou *et al* 1995, *Virology* **214** 167-176) and can be characterized by electron microscopy and DNA blotting. The infectivity of BPV particles isolated from the cultured cells may be tested in focus formation assays using C127 fibroblasts.

EXAMPLE 9

Method for extracting and measuring tRNA from tissues

Tissue(100g) is homogenized in a Waring Blender with 150 mL of phenol (Mallinckrodt, Analytical Reagent, 88%) saturated with water (15:3) and 150 mL of 1.0 M NaCl, 0.005 M EDTA in 0.1 M Tris-chloride buffer, pH 7.5. The homogenate was spun for ten minutes at top speed in the International clinical centrifuge and the upper layer was carefully decanted off. To this aqueous layer, three volumes of 95% ethanol were added. The resultant precipitate was spun down at top speed in the International clinical centrifuge and resuspended in 250 mL of 0.1 M Tris/chloride buffer, pH 7.5. This solution was added (flow rate of 15-20 drops per minute) to a column (2 x 10 cm) of 2 g of DEAE-cellulose previously equilibrated with cold 0.1 M Tris-chloride buffer pH 7.5. The column was then washed with 1 L of Tris-chloride buffer, pH 7.5 and the RNA eluted with 1.0 M NaCl in 0.1 M Tris-chloride buffer, pH 7.5. The first 10 mL of NaCl solution were discarded as "hold-up." Sufficient salt solution (60-80 mL) was then collected until the optical density of the effluent was less than three at 260 nm. This solution was extracted twice with an equal volume of phenol saturated with water and twice with ether. To the aqueous solution containing the RNA, three volumes of 95% ethanol were added and the solution wag allowed to stand overnight in the cold. The precipitate was spun down and washed first with 80% and then twice with 95% ethanol and

dried in a vacuum. Approximately 60 mg of soluble RNA were obtained from a 100-g lot of rat liver.

Quantitating tRNAs

The following nylon membranes are used: Biodine A and B (PALL). For the preparation of dot blots, the tRNA samples (from 1 pg to 5 ng) are denatured at 60 °C for 15 min in 1-5 μL of 15% formaldehyde. 10x SSC (SSC is NaCl 0.3 M, tri-sodium citrate 0.03 M). The samples are spotted in 1 μL aliquots onto the membranes that have been soaked for 15 min in deionized water and slightly dried between two sheets of 3MM Whatman paper prior to the application of the samples. The tRNAs are fixed covalently (in the membranes by ultraviolet-irradiation (10 mm using an ultraviolet lamp at 254 nm and 100 W strength at a distance of 20 cm) and the membranes are baked for 2-3 h at 80 °C.

A 5' end labelled synthetic deoxyribo-oligonucleotide complementary to the A54-A73 sequence of the tRNA is used as a probe for the hybridization experiments. Labelling of the oligonucleotide is performed by direct phosphorylation of the 5' OH' ended probe.

For hybridisation experiments, the UV-irradiated membranes are first preincubated for 5 h at 50 C in 50% deionized formamide, 5 x SSC, 1% SDS, 0.04% Ficoll 0.04% polyvinylpyrrolidone and 250 μL/mL of sonicated salmon sperm DNA using 5 mL of buffer for 100 cm^2 of membrane. Hybridization is finally performed overnight at 50 °C in the above solution (2.5 mL/100 cm^2) where the labeled probe has been

added. After hybridization, the membranes are washed twice in 2 x SSC, 0.1% SDS for 5 min at room temperature, twice in 2 x SSC, 1% SDS for 30 mm at 60 °C and finally in 0.1 x SSC. 0.1% SDS for 30 min at room temperature. To detect the hybridized probes the membranes are exposed for 16 h to Fuji XR film at 70 °C with an intensifying screen.

Sequence of tRNA probes

The sequences of the tRNA probes are as follows:

Ala^{GCA}:	5'-TAAGGACTGTAAGACTT	(SEQ ID NO:13)
Arg^{CGA}:	5'-CGAGCCAGCCAGGAGTC	(SEQ ID NO:14)
Asn^{AAC}:	5'-CTAGATTGGCAGGAATT	(SEQ ID NO:15)
Asp^{GAC}:	5'-TAAGATATATAGATTAT	(SEQ ID NO:16)
Csy^{TGC}:	5'-AAGTCTTAGTAGAGATT	(SEQ ID NO:17)
Glu^{GAA}:	5'-TATTTCTACACAGCATT	(SEQ ID NO:18)
Gln^{CAA}:	5'-CTAGGACAATAGGAATT	(SEQ ID NO:19)
Gly^{GGA}:	5'-TACTCTCTTCTGGGTTT	(SEQ ID NO:20)
His^{CAC}:	5'-TGCCGTGACTCGGATTC	(SEQ ID NO:21)
Ile^{ATC}:	5'-TAGAAATAAGAGGGCTT	(SEQ ID NO:22)
Leu^{CTA}:	5'-TACTTTTATTTGGATTT	(SEQ ID NO:23)
Leu^{CTT}:	5'-TATTAGGGAGAGGATTT	(SEQ ID NO:24)
Lys^{AAA}:	5'-TCACTATGGAGATTTTA	(SEQ ID NO:25)
Lys^{AAG}:	5'-CGCCCAACGTGGGGCTC	(SEQ ID NO:26)
Met^{elong}	5'-TAGTACGGGAAGGATTT	(SEQ ID NO:27)
Phe^{TTC}:	5'-TGTTTATGGGATACAAT	(SEQ ID NO:28)
Pro^{CCA}:	5'-TCAAGAAGAAGGAGCTA	(SEQ ID NO:29)
Pro^{CCI}:	5'-GGGCTCGTCCGGGATTT	(SEQ ID NO:30)
Ser^{AGC}:	5'-ATAAGAAAGGAAGATCG	(SEQ ID NO:31)
Thr^{ACA}:	5'-TGTCTTGAGAAGAGAAG	(SEQ ID NO:32)
Tyr^{TAC}:	5'-TGGTAAAAAGAGGATTT	(SEQ ID NO:33)

Val^{GTA}: 5'-TCAGAGTGTTCATTGGT (SEQ ID NO:34)

EXAMPLE 10

Comparison of the relative abundance of tRNA species in undifferentiated and differentiated keratinocytes

Materials and Methods

Isolation of epidermal cells

2-day old mice were killed and their skins removed. The skins were digested with 0.25% trypsin PBS at 4 °C overnight. The epidermis was separated from the dermis using forceps and minced with scissors in 10% FCS DMEM medium. The cell suspension was first filtered through a 1 mm and then a 0.2 mm nylon net. The cell suspension was then pelleted and washed twice with PBS.

Density gradient centrifugation

The keratinocytes were resuspended in 30% Percoll and separated by centrifugation through a discontinuous Percoll gradient (1.085, 1.075 and 1.050 g/mL) at 1200 x g at room temperature for 25 min. The cells were then washed with PBS and used to extract tRNA.

tRNA purification

The cells were lysed in 5 mL of lysis buffer (0.2 M NaOH, 1% SDS) for 10 min at room temperature. The lysate was neutralized with 5 mL of 3.0 M potassium acetate (pH 5.5). After centrifugation, the supernatant was diluted with 3

volumes of 100 mM Tris (pH 7.5) and added to a DEAE column equilibrated with 100 mM Tris (pH 7.5). An equal volume of isopropanol was added to the aqueous solution containing tRNA, and the solution was allowed to stand overnight at 4 °C. The tRNA was spun down and washed with 75% ethanol, then dissolved in RNase-free water.

tRNA blotting

10 ng of each tRNA sample in 1 μL was denatured in 60°C for 15 min in 4 μL formaldehyde and 5 μL 20 x SSC. The samples were spotted in 1 μL aliquots onto charged nylon membrane (Amersham), and the tRNAs were fixed with UV and probed with ^{32}P-oligonucleotides.

Results

Comparison of the abundance of the tRNA species in undifferentiated and differentiated keratinocytes showed that the levels of some tRNA populations changed dramatically. For example, the levels of tRNAs specific for Ala^{GCA}, Leu^{CTT}, Leu^{CTA} were increased in differentiated cells while tRNAs for Arg^{CGA}, Pro^{CCI}, Asn^{AAG} were more abundant in undifferentiated keratinocytes (see Table 2).

GENERAL DISCUSSION

In the present specification the inventors have confirmed that one determinant of the efficiency of translation of a gene in mammalian cells is its codon composition. This observation has commonly been

made when genes from prokaryotic organisms have been expressed in eukaryotic cells (Smith, D. W., 1996, *Biotechnol. Prog.* **12**:417-422). The present inventors have also presented evidence that mRNA encoding the capsid genes of papillomavirus are not effectively translated in cultured eukaryotic cells, apparently because tRNA availability is rate limiting for translation, and that the block to PV late gene translation in eukaryotic cells in culture can be overcome by altering the codon usage of the late genes to match the consensus for mammalian genes, or alternatively by providing exogenous tRNAs. Alterations to mRNA secondary structure or protein binding (Sokolowski, *et al.*, 1998, *J. Virol.* **72**:1504-1515) as a consequence of the changes to the primary sequence of the PV capsid genes might contribute to the observed differences in efficiency of translation of the natural and modified PV capsid gene mRNAs in cultured cells. However, the enhancement of translation of the natural but not the modified mRNA that was observed after addition of tRNA in a mammalian *in vitro* translation system, which was not observed in a plant translation system, strengthens the argument that tRNA availability is rate limiting for translation of the natural gene in mammalian cells. A shortage of critical tRNAs could result in slowed elongation of the nascent peptide or premature termination of translation (Oba, *et al.*, 1991, *Biochimie* **73**:1109-1112). Slowed elongation appears to be the major consequence for the PV late gene. Analysis of codon usage in the PV genome shows that PV late genes use many codons that mammalian cells rarely

use. For example, PV frequently uses UUA for leucine, CGU for arginine, ACA for threonine, and AUA for isoleucine, whereas these codons are significantly less often used in mammalian genes. In contrast, papillomavirus late genes can be expressed efficiently in yeast (Jansen, *et al.*, 1995, *Vaccine* **13**:1509-1514) (Sasagawa, *et al.*, 1995, *Virology* **206**:126-135) and the codon composition of yeast and papillomavirus genes are similar (Table 1). An apparent exception is that PV L1 genes can be efficiently expressed in insect cells (Kirnbauer, *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* **89**:12180-12184) using recombinant baculovirus, or in various undifferentiated mammalian cells using recombinant vaccinia (Zhou, *et al.*, 1991, *Virology* **185**:251-257). As infection with vaccinia or baculovirus down regulates cellular protein synthesis, the efficient expression of the L1 capsid proteins under these circumstances may occur because less cellular mRNA is available in a virus infected cell to compete with the L1 mRNA for the rarer tRNAs.

Codon composition could be a more general determinant of gene expression within different stages of differentiation of the same tissue. Although the genetic code is essentially universal, different organisms show differences in codon composition of their genes, while the codon composition of genes tends to be relatively similar for all genes within each organism, and matched to the population of iso-tRNAs for that organism (Ikemura, T., 1981, *J. Mol. Biol.* **146**:1-21). However, populations of tRNAs in differentiating and neoplastic cells are different (Kanduc, D., 1997, *Arch. Biochem. Biophys.* **342**:1-6;

Yang, and Comb, 1968, *J. Mol. Biol.* **31**:138-142; Yang, and Novelli, 1968, *Biochem. Biophys. Res. Commun.* **31**: 534-539) and the tRNA populations also vary in cells growing under different growth conditions (Doi, *et al.*, 1968, *J. Biol. Chem.* **243**:945-951). Accordingly, the inventors believe that codon composition and tRNA availability together provide a primitive mechanism for spatial and/or temporal regulation of gene expression. It is well recognized that the G+C content of many dsDNA viruses, a crude marker for viral gene codon composition, is markedly different from the G+C content of the DNA of the cells they infect (Strauss, *et al.*, 1995, "Virus Evolution" *in* Virology (eds. Fields, B. N., *et al.*), Lipipincott-Raven, Philadelphia, pp 153-171). Viruses may therefore have evolved to take advantage of codon composition to regulate their own program of gene expression, perhaps to avoid expression of lethal quantities of viral proteins in undifferentiated cells where the virus utilizes the cellular machinery to replicate its genome.

As the inventors' observations represent an apparently novel mechanism of regulation of gene translation within a single tissue, it is relevant to consider how this relates to previously proposed hypotheses for the restriction of expression of PV late genes to differentiated epithelium. A number of explanations have been proposed for the observation that PV late genes are only effectively expressed in differentiated epithelium. Reduced late gene transcription may reflect dependence of transcription from the late promoter on transcription factors

expressed only in differentiated epithelium, or may alternatively be due to suppression of late promoter transcription by viral (Stubenrauch, *et al.*, 1996, *J. Virol.* **70**:119-126) or cellular gene products expressed in undifferentiated cells. The "late" promoters of HPV31b and of HPV5 (Haller, *et al.*, 1995, *Virology* **214**:245-255; Hummel, *et al.*, 1992, *J. Virol.* **66**:6070-6080) are described as differentiation dependent, although the search for relevant transcription control factors in differentiated keratinocytes by conventional footprinting and DNA binding studies has to date been unrewarding. Our data show that capsid proteins are not translated from PV L1 and L2 mRNAs in cells transfected with CMV promoter-based expression vectors (Fig. 2), suggesting that in addition to any transcriptional controls that may exist that there is a post-transcriptional block to capsid protein synthesis in undifferentiated cells. Sequences resembling 5' splice donor sites exist within L1 or L2 mRNA or within flanking untranslated message which are inhibitory to transcription of genes with which they are associated (Kennedy, *et al.*, 1991, *J. Virol.* **65**:2093-2097) (Furth, *et al.*, 1994, *Mol. Cell. Biol.* **14**:5278-5289). Other AU rich sequences in L1 or L2 mRNA promote mRNA degradation (Sokolowski, *et al.*, 1997, *Oncogene* **15**:2303-2319). These mechanisms inhibiting L1 and L2 expression in undifferentiated cells have yet to be shown to be inactive in differentiated epithelium, to explain the successful translation of late genes in this tissue.

Because inhibitory RNA sequences within the L1 coding sequence could have been rendered non-

functional by the systematic codon substitution employed in the experiments described herein and the untranslated inhibitory sequences were not included in the inventors' test system, the respective roles of inhibitory sequences and codon mismatch in suppression of PV late gene expression in cultured mammalian cells cannot be determined. However, regulatory sequences promoting RNA degradation or inhibiting translation are presumed to act through interaction with nuclear or cytoplasmic proteins (Sokolowski, *et al.*, 1998, *J. Virol.* **72**:1504-1515), and inefficient translation of native sequence L1 mRNA was observed in a cell free translation system from anucleate cells, demonstrating that codon composition of the PV late genes must play some role in regulation of PV late gene translation.

Further evidence supporting the hypothesis that codon composition is an important determinant of PV capsid gene expression was gathered from an analysis of the 84 PV L1 sequences currently available in Genebank. The codon composition of the L1 genes, and particularly the frequency of usage of the rarer codons, was essentially the same across all the published sequences (data not shown) as would be predicted by the similar G+C content of the papillomavirus genomes. The PV L1 gene is relatively conserved at the amino acid level, showing 60 - 80% amino acid homology between PV genotypes, as might be expected by the constraints on capsid protein function. There are, however, no obvious constraining influences on the codon composition of the PV late genes beyond those of the inventors' hypothesis, as the late gene region does not code for other genes,

either in other reading frames or on the complementary DNA strand, and has no known *cis* acting regulatory functions. If codon composition of the capsid genes were not important for PV function, a considerable heterogeneity of codon usage might therefore be expected, given the evolutionary diversity of PVs (Chan, *et al.* 1995, *J. Virol.* **69**:3074-3083).

Taken together, the data and evidence outlined herein makes a strong case that codon usage is a significant determinant of expression of PV late genes in undifferentiated and differentiated epithelial cells, and that this observation is generalizable. The relative role of message instability and codon mismatch in determining expression in differentiated tissues will require comparisons of transcriptional activity and translation of the L1 or L2 genes driven from strong constitutive promoters in differentiated and undifferentiated epithelium. Such work should now be feasible using either transgenic technology or keratinocyte raft cultures.

Although mechanisms of transcriptional regulation of PV L1 or L2 gene expression in the superficial layer of differentiated epithelium have been proposed (Zeltner *et al.*, 1994, *J. Virol.* **68**:3620; Brown, *et al.*, 1995, *Virology* **214**:259; Stoler *et al.*, 1992, *Hum. Pathol.* **23**:117; Hummel *et al.*, 1995, *J. Virol.* **69**:3381; Haller *et al.*, 1995, *Virology* **214**:245; Barksdale and Baker, 1993, *J. Virol.* **67**:5605), measurable PV late gene mRNA is not always associated with production of late proteins (Zeltner *et al.*, 1994, *supra*; Ozbun and Meyers, 1997, *J. Virol.*

71:5161), and the data presented here suggest that translation regulation may play a major part in controlling PV late gene expression. This observation has implications as herein described for the regulation of expression of genes related to the specialised functions of any differentiated tissue, and also for targeting of expression of therapeutic genes to such tissue while avoiding the potentially deleterious consequences of expression of the exogenous gene in a self renewing stem cell population.

The present invention has been described in terms of particular embodiments found or proposed by the present inventors to comprise preferred modes for the practice of the invention. Those of skill in the art will appreciate that, in light of the present disclosure, numerous modifications and changes may be made in the particular embodiments exemplified without departing from the scope of the invention. All such modifications are intended to be included within the scope of the appended claims.

TABLE LEGENDS

TABLE 1

The codon usage data for human, cow yeast and wheat proteins are derived from published results(18). The BPV1 data are from the sequences in the Genbank database.

TABLE 2

Each iso-acceptor tRNA with anticodon shown as superscript are shown on top row. The "+" indicates the abundance of tRNA wherein each "+" indicates about 10 fold increase.

TABLES

TABLE 1

Frequency (per one thousand) of codon usage for individual organisms.

Amino acids	Codons	Human	Cow	Yeast	Wheat	BPVL1/ L2
ARG	CGA	5.4	5.5	2.3	2.3	7.2
	CGC	11.3	12.2	2.0	7.5	4.1
	CGG	10.4	11.2	1.1	4.6	5.1
	CGU	4.7	3.7	7.5	1.1	10.4
	AGA	9.9	9.9	24.0	4.1	14.4
	AGG	11.1	11.4	7.5	7.1	9.3
LEU	CUA	6.2	4.9	11.8	12.1	18.6
	CUC	19.9	21.2	4.1	18.6	6.2
	CUG	42.5	46.6	8.3	15.5	15.5
	CUU	10.7	10.6	9.6	6.5	20.7
	UUA	5.3	4.0	24.5	1.8	14.5
	UUG	11.0	9.6	32.1	15.3	15.5
SER	UCA	9.3	7.6	15.6	14.6	16.6
	UCC	17.7	17.6	14.4	10.1	11.4
	UCG	4.2	4.5	6.5	9.6	6.2
	UCU	13.2	11.2	24.6	14.8	15.5
	AGC	18.7	18.7	7.1	12.8	12.4
	AGU	9.4	8.6	11.7	12.9	21.7
THR	ACA	14.4	11.4	15.6	4.6	37.3
	ACC	23.0	21.1	13.9	15.9	19.7
	ACG	6.7	7.8	6.7	4.5	4.1
	ACU	12.7	9.6	22.0	11.8	28.0

Amino acids	Codons	Human	Cow	Yeast	Wheat	BPVL1/ L2
PRO	CCA	14.6	12.0	21.4	71.2	22.8
	CCC	20.0	19.2	5.9	11.1	15.5
	CCG	6.5	7.9	4.1	19.4	0.0
	CCU	15.5	14.6	12.8	10.3	33.1
ALA	GCA	14.0	13.1	15.3	11.2	33.1
	GCC	29.1	35.8	15.5	19.5	17.6
	GCG	7.2	9.3	5.1	13.8	4.1
	GCU	19.6	19.1	28.3	9.6	13.5
GLY	GGA	17.1	16.2	8.9	25.9	22.8
	GGC	25.4	28.1	8.9	28.0	12.4
	GGG	17.3	19.2	5.1	28.5	22.8
	GGU	11.2	11.8	34.9	9.6	18.6
VAL	GUA	5.9	5.1	10.0	4.4	15.5
	GUC	16.3	18.4	14.9	14.8	6.2
	GUG	30.9	32.9	9.5	12.9	23.8
	GUU	10.4	9.9	26.6	11.6	16.6
LYS	AAA	22.2	21.6	37.7	4.5	37.2
	AAG	34.9	37.1	35.2	17.4	13.5
ASN	AAC	22.6	22.4	25.8	14.2	10.3
	AAU	16.6	12.5	31.4	6.7	24.8
GLN	CAA	11.1	9.7	29.8	171.8	22.8
	CAG	33.6	34.4	10.4	79.4	17.6
HIS	CAC	14.2	14.0	8.2	8.2	6.2
	CAU	9.3	7.5	12.3	7.1	13.4
GLU	GAA	26.8	24.4	48.9	7.8	36.2
	GAG	41.4	45.4	16.9	19.7	21.7
ASP	GAC	29.0	31.5	22.3	13.0	18.6
	GAU	21.7	19.2	37.0	4.0	33.1

Amino acids	Codons	Human	Cow	Yeast	Wheat	BPVL1/ L2
TYR	UAC	18.8	20.3	16.5	24.5	17.6
	UAU	12.5	10.5	16.5	12.5	18.6
CYS	UGC	14.5	13.9	3.7	14.8	5.2
	UGU	9.9	9.4	7.6	4.9	5.2
PHE	UUC	22.6	25.5	20.0	14.1	7.2
	UUU	15.8	17.0	23.2	15.0	23.8
ILE	AUA	5.8	5.2	12.8	5.4	22.7
	AUC	24.3	25.8	18.4	19.7	8.2
	AUU	14.9	13.1	31.1	10.7	20.7

TABLE 2

tRNA population changes as KC starts to differentiate.

tRNA	Arg^{CGA}	Ala^{GCA}	His^{CAC}	Leu^{CTT}	Leu^{CTA}	Lys^{AAG}	Lys^{AAA}	Met^{Ini}	Pro^{CCI}
Supra	+	+++	+	+++	+++	++	+	+	+
Basal	+++	+	++	+	+	+	+	++	+++
tRNA	Val^{GTA}	Val^{GTI}	His^{CAC}	Asn^{AAG}	Thr^{ACA}	Met^{Elo}	Gly^{GGA}		
Supra	++	+	++	+	+	+	+		
Basal	+	+	+	+++	+	++	+		

WHAT IS CLAIMED IS:

1. A synthetic nucleic acid sequence capable of selectively expressing a protein in a target cell or tissue of a mammal, wherein said selective expression is effected by replacing at least one existing codon of a parent nucleic acid sequence with a synonymous codon to form said synthetic nucleic acid sequence.

2. The nucleic acid sequence of claim 1, wherein said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to one or more other cells or tissues of the mammal.

3. The nucleic acid sequence of claim 1, wherein said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to a precursor cell or tissue.

4. The nucleic acid sequence of claim 1, wherein said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to a cell or tissue derived therefrom.

5. The nucleic acid sequence of claim 1, wherein said synonymous codons for selective expression of said protein are selected from the group consisting of gca (Ala), cuu (Leu) and cua (Leu), and said target is a differentiated cell.

6. The nucleic acid sequence of claim 5, wherein said differentiated cell is a differentiated keratinocyte.

7. The nucleic acid sequence of any one of claims 2 to 4, wherein said corresponding iso-tRNA in said target cell or tissue is at a level which is at least 110%, preferably at least 200%, more preferably at least 500%, and most preferably at least 1000%, of that expressed in the or each other cell or tissue of the mammal.

8. The nucleic acid sequence of claim 1, wherein the synonymous codon may be selected from the group consisting of (1) a codon used at relatively high frequency by genes, preferably highly expressed genes, of the target cell or tissue, (2) a codon used at relatively high frequency by genes, preferably highly expressed genes, of the or each other cell or tissue, (3) a codon used at relatively high frequency by genes, preferably highly expressed genes, of the mammal, (4) a codon used at relatively low frequency by genes of the target cell or tissue, (5) a codon used at relatively low frequency by genes of the or each other cell or tissue, (6) a codon used at relatively low frequency by genes of the mammal, (7) a codon used at relatively high frequency by genes of another organism, and (8) a codon used at relatively low frequency by genes of another organism.

9. The nucleic acid sequence of claim 1, wherein the at least one existing codon and the synonymous codon are selected such that said protein is expressed from said synthetic nucleic acid sequence in said target cell or tissue at a level which is at

least 110%, preferably at least 200%, more preferably at least 500%, and most preferably at least 1000%, of that expressed from said parent nucleic acid sequence in said target cell or tissue.

10. A method for selectively expressing a protein in a target cell or tissue of a mammal, wherein said selective expression is effected by replacing at least one existing codon of a parent nucleic acid sequence with a synonymous codon to form said synthetic nucleic acid sequence.

11. The method of claim 10, wherein said method is further characterized the steps of:

(a) replacing at least one existing codon of a parent nucleic acid sequence encoding said protein with a synonymous codon to produce a synthetic nucleic acid sequence having altered translational kinetics compared to said parent nucleic acid sequence such that said protein is selectively expressible in said target cell or tissue;

(b) administering to the mammal and introducing into said target cell or tissue, or a precursor cell or precursor tissue thereof, said synthetic nucleic acid sequence operably linked to one or more regulatory nucleotide sequences; and

(c) selectively expressing said protein in said target cell or tissue.

12. The method of claim 11 further including, prior to step (a):

(i) measuring relative abundance of different iso-tRNAs in said target cell or tissue, and in one or more other cells or tissues of the mammal; and

(ii) identifying said at least one existing codon and said synonymous codon based on said measurement, wherein said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the existing codon, is in higher abundance in said target cell or tissue relative to the or each other cell or tissue of the mammal.

13. The method of claim 12, wherein step (ii) is further characterized in that said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to a precursor cell or tissue.

14. The method of claim 12, wherein step (ii) is further characterized in that said synonymous codon corresponds to an iso-tRNA which, when compared to an iso-tRNA corresponding to the at least one existing codon, is in higher abundance in the target cell or tissue relative to a cell or tissue derived therefrom.

15. The method of claim 11 further including, prior to step (a), identifying said at least one existing codon and said synonymous codon based on respective relative frequencies of particular codons used by genes selected from the group consisting of (I) genes of the target cell or tissue, (II) genes of the or each other cell or tissue, (III) genes of the mammal, and (IV) genes of another organism.

16. A method for expressing a protein in a target cell or tissue from a first nucleic acid sequence including the steps of:

introducing into said target cell or tissue, or a precursor cell or precursor tissue

thereof, a second nucleic acid sequence encoding at least one isoaccepting transfer RNA wherein said second nucleic acid sequence is operably linked to one or more regulatory nucleotide sequences, and wherein said at least one isoaccepting transfer RNA is normally in relatively low abundance in said target cell or tissue and corresponds to a codon of said first nucleic acid sequence.

17. A method for producing a virus particle in a cycling eukaryotic cell, said virus particle comprising at least one protein necessary for assembly of said virus particle, wherein said at least one protein is not expressed in said cell from a parent nucleic acid sequence at a level sufficient to permit virus assembly therein, said method including the steps of:

(a) replacing at least one existing codon of said parent nucleic acid sequence with a synonymous codon to produce a synthetic nucleic acid sequence having altered translational kinetics compared to said parent nucleic acid sequence such that said at least one protein is expressible from said synthetic nucleic acid sequence in said cell at a level sufficient to permit virus assembly therein;

(b) introducing into said cell or a precursor thereof said synthetic nucleic acid sequence operably linked to one or more regulatory nucleotide sequences; and

(c) expressing said at least one protein in said cell in the presence of other viral proteins required for assembly of said virus particle to thereby produce said virus particle.

18. A method for producing a virus particle in a cycling cell, said virus particle comprising at least one protein necessary for assembly of said virus particle, wherein said at least one protein is not expressed in said cell from a parent nucleic acid sequence at a level sufficient to permit virus assembly therein, and wherein at least one existing codon of said parent nucleic acid sequence is rate limiting for the production said at least one protein to said level, said method including the step of introducing into said cell a nucleic acid sequence capable of expressing therein an isoaccepting transfer RNA specific for said at least one codon.

19. A vector comprising a nucleic acid sequence according to any of claims 1 to 9 wherein said synthetic nucleic acid sequence is operably linked to one or more regulatory nucleic acid sequences.

20. A pharmaceutical composition comprising a nucleic acid sequence according to any of claims 1 to 9 together with a pharmaceutically acceptable carrier.

21. A pharmaceutical composition comprising a vector according to claim 19 together with a pharmaceutically acceptable carrier.

22. A cell or tissue comprising therein a nucleic acid sequence according to any of claims 1 to 9.

23. A cell or tissue comprising therein a vector according to claim 19.

24. A cell or tissue resulting from a method according to any one of claims 10 to 18.

25. Virus particles produced from a method according to claims 17 or 18.

Figure 1A

```
          C C         G   G                   C   G   C   T       C
1     ATG GCG TTG TGG CAA CAA GGC CAG AAG CTG TAT CTC CCT CCA ACC CCT
      M   A   L   W   Q   Q   G   Q   K   L   Y   L   P   P   T   P
        G
49    GTA AGC AAG GTG CTT TGC AGT GAA ACC TAT GTG CAA AGA AAA AGC ATT
      V   S   K   V   L   C   S   E   T   Y   V   Q   R   K   S   I
                                        G   C   C       C   C
97    TTT TAT CAT GCA GAA ACG GAG CGC CTG CTA ACT ATA GGA CAT CCA TAT
      F   Y   H   A   E   T   E   R   L   L   T   I   G   H   P   Y
          C       C               G       G           G   C   C
145   TAC CCA GTG TCT ATC GGG GCC AAA ACT GTT CCT AAG GTC TCT GCA AAT   (SauI)
      Y   P   V   S   I   G   A   K   T   V   P   K   V   S   A   N
                  G   C       C       G
193   CAG TAT AGG GTA TTT AAA ATA CAA CTA CCT GAT CCC AAT CAA TTT GCA
      Q   Y   R   V   F   K   I   Q   L   P   D   P   N   Q   F   A
        G               C   G           C   C
241   CTA CCT GAC AGG ACT GTT CAC AAC CCA AGT AAA GAG CGG CTG GTG TGG   (BalI)
      L   P   D   R   T   V   H   N   P   S   K   E   R   L   V   W
            G   C   C                       C           G   C   C   C
289   CCA GTC ATA GGT GTG CAG GTG TCC AGA GGG CAG CCT CTT GGA GGT ACT
      P   V   I   G   V   Q   V   S   R   G   Q   P   L   G   G   T
        G
```

Figure 1A cont'd

```
337  GTA ACT GGG CAC CCC ACT TTT AAT GCT TTG CTT GAT GCA GAA AAT GTG
      V   T   G   H   P   T   F   N   A   L   L   D   A   E   N   V
                          G   C   C   C               G

385  AAT AGA AAA GTC ACC ACC CAA ACA ACA GAT GAC AGG AAA CAA ACA GGC   (StuI)
      N   R   K   V   T   T   Q   T   T   D   D   R   K   Q   T   G
       G       C       G   G       C       C   G

433  CTA GAT GCT AAG CAA CAA CAG ATT CTG TTG CTA GGC TGT ACC CCT GCT
      L   D   A   K   Q   Q   Q   I   L   L   L   G   C   T   P   A
                                                G   C   C

481  GAA GGG GAA TAT TGG ACA ACA GCC CGT CCA TGT GTT ACT GAT CGT CTA   (XbaI)
      E   G   E   Y   W   T   T   A   R   P   C   V   T   D   R   L
          C                       G   G C G                   C

529  GAA AAT GGC GCC TGC CCT CCT CTT GAA TTA AAA AAC AAG CAC ATA GAA
      E   N   G   A   C   P   P   L   E   L   K   N   K   H   I   E

577  GAT GGG GAT ATG ATG GAA ATT GGG TTT GGT GCA GCC AAC TTC AAA GAA
      D   G   D   M   M   E   I   G   F   G   A   A   N   F   K   E
```

Figure 1A cont'd

```
                                                  G       C
625   ATT AAT GCA AGT AAA TCA GAT CTA CCT CTT GAC ATT CAA AAT GAG ATC
       I   N   A   S   K   S   D   L   P   L   D   I   Q   N   E   I
           C       C           G                       C   C   C   C
673   TGC TTG TAC CCA GAC TAC CTC AAA ATG GCT GAG GAC GCT GCT GGT AAT   (NaeI)
       C   L   Y   P   D   Y   L   K   M   A   E   D   A   A   G   N
                   C   C   C       G   G           C   G
721   AGC ATG TTC TTT TTT GCA AGG AAA GAA CAG GTG TAT GTT AGA CAC ATC
       S   M   F   F   F   A   R   K   E   Q   V   Y   V   R   H   I
                   C       C
769   TGG ACC AGA GGG GGC TCG GAG AAA GAA GCC CCT ACC ACA GAT TTT TAT
       W   T   R   G   G   S   E   K   E   A   P   T   T   D   F   Y
         G       C   C   G   C   C           G   G   C       C       C
817   TTA AAG AAT AAT AAA GGG GAT GCC ACC CTT AAA ATA CCC AGT GTG CAT
       L   K   N   N   K   G   D   A   T   L   K   I   P   S   V   H
         C   C   C       C                   G   C   C   C   C   G   C   C
865   TTT GGT AGT CCC AGT GGC TCA CTA GTC TCA ACT GAT AAT CAA ATT TTT   (SpeI)
       F   G   S   P   S   G   S   L   V   S   T   D   N   Q   I   F
         C                       G       C
913   AAT CGG CCC TAC TGG CTA TTC CGT GCC CAG GGC ATG AAC AAT GGA ATT
       N   R   P   Y   W   L   F   R   A   Q   G   M   N   N   G   I
```

Figure 1A cont'd

```
        C       C   C C G C     C C G   C       C       C           C
961     GCA TGG AAT AAT TTA TTG TTT TTA ACA GTG GGG GAC AAT ACA CGT GGT   (BsaAI)
        A   W   N   N   L   L   F   L   T   V   G   D   N   T   R   G
        C   C   G       C   C   G       C                   G   C
1009    ACT AAT CTT ACC ATA AGT GTA GCC TCA GAT GGA ACC CCA CTA ACA GAG
        T   N   L   T   I   S   V   A   S   D   G   T   P   L   T   E
                G           C   G       C       C       G   G
1057    TAT GAT AGC TCA AAA TTC AAT GTA TAC CAT AGA CAT ATG GAA GAA TAT
        Y   D   S   S   K   F   N   V   Y   H   R   H   M   E   E   Y
                C   C C G       G       C       G       C   C   G
1105    AAG CTA GCC TTT ATA TTA GAG CTA TGC TCT GTG GAA ATC ACA GCT CAA   (NheI)
        K   L   A   F   I   L   E   L   C   S   V   E   I   T   A   Q
        C       C                   G           C       G   G
1153    ACT GTG TCA CAT CTG CAA GGA CTT ATG CCC TCT GTG CTT GAA AAT TGG
        T   V   S   H   L   Q   G   L   M   P   S   V   L   E   N   W
        G   C   C           C   C               C   G               C
1201    GAA ATA GGT GTG CAG CCT CCT ACC TCA TCG ATA TTA GAG GAC ACC TAT   (ClaI)
        E   I   G   V   Q   P   P   T   S   S   I   L   E   D   T   Y
            C   C       C   C   C   C   G       C       C   G
1249    CGC TAT ATA GAG TCT CCT GCA ACT AAA TGT GCA AGC AAT GTA ATT CCT
        R   Y   I   E   S   P   A   T   K   C   A   S   N   V   I   P
```

Figure 1A cont'd

```
                                                        C           C   C   G
1297  GCA AAA GAA GAC CCT TAT GCA GGG TTT AAG TTT TGG AAC ATA GAT CTT
       A   K   E   D   P   Y   A   G   F   K   F   W   N   I   D   L
        G   G       G       C       C G       G   C           C
1345  AAA GAA AAG CTT TCT TTG GAC TTA GAT CAA TTT CCC TTG GGA AGA AGA   (StyI)
       K   E   K   L   S   L   D   L   D   Q   F   P   L   G   R   R
           C G   C       G       C   C       C   C               C   G
1393  TTT TTA GCA CAG CAA GGG GCA GGA TGT TCA ACT GTG AGA AAA CGA AGA
       F   L   A   Q   Q   G   A   G   C   S   T   V   R   K   R   R
        C       G   G   C       C       C   C   G   G   G       G
1441  ATT AGC CAA AAA ACT TCC AGT AAG CCT GCA AAA AAA AAA AAA AAA TAA
       I   S   Q   K   T   S   S   K   P   A   K   K   K   K   K   -
```

Figure 1B

```
                C   C   C   G           G   G   C           C
    1   ATG AGT GCA CGA AAA AGA GTA AAA CGT GCC AGT GCC TAT GAC CTG TAC
          M   S   A   R   K   R   V   K   R   A   S   A   Y   D   L   Y
                  C                                       C
   49   AGG ACC TGC AAG CAA GCG GGC ACA TGT CCA CCA GAT GTG ATA CGA AAG
          R   T   C   K   Q   A   G   T   C   P   P   D   V   I   R   K
            G   G   C   C   C   C   C   C   G   C C     G   C   C   C   G
   97   GTA GAA GGA GAT ACT ATA GCA GAT AAA ATT TTG AAA TTT GGG GGT CTT
          V   E   G   D   T   I   A   D   K   I   L   K   F   G   G   L
            C           C G   C   C   G   C   C                 C   C
  145   GCA ATC TAC TTA GGA GGG CTA GGA ATA GGA ACA TGG TCT ACT GGA AGG   (AccI)
          A   I   Y   L   G   G   L   G   I   G   T   W   S   T   G   R
            G   C   C   C   C                   C       G   C   C   C   C
  193   GTG GCC GCA GGT GGA TCA CCA AGG TAC ACA CCA CTC CGA ACA GCA GGG
          V   A   A   G   G   S   P   R   Y   T   P   L   R   T   A   G
                C   C   C   G   C   C   C                   C   G   C   C
  241   TCC ACA TCA TCG CTT GCA TCA ATA GGA TCC AGA GCT GTA ACA GCA GGG   (BamHI)
          S   T   S   S   L   A   S   I   G   S   R   A   V   T   A   G
```

Figure 1B cont'd

```
                TCC   C   C           C     C G           G
289  ACC CGC CCC AGT ATA GGT GCG GGC ATT CCT TTA GAC ACC CTT GAA ACT
      T   R   P   S   I   G   A   G   I   P   L   D   T   L   E   T
                C     C   T   C       C           C       G   C   A
337  CTT GGG GCC TTG CGT CCA GGG GTG TAT GAG GAC ACT GTG CTA CCA GAG
      L   G   A   L   R   P   G   V   Y   E   D   T   V   L   P   E
              C   C   G   C       C   C   G           C   C   C   G
385  GCC CCT GCA ATA GTC ACT CCT GAT GCT GTT CCT GCA GAT TCA GGG CTT   (PstI)
      A   P   A   I   V   T   P   D   A   V   P   A   D   S   G   L
       C                C   C           C       C           G   C   C
433  GAT GCC CTG TCC ATA GGT ACA GAC TCG TCC ACG GAG ACC CTC ATT ACT
      D   A   L   S   I   G   T   D   S   S   T   E   T   L   I   T
          G                C       A           C   G   G   A   C   G
481  CTG CTA GAG CCT GAG GGT CCC GAG GAC ATA GCG GTT CTT GAG CTG CAA   (SauI)
      L   L   E   P   E   G   P   E   D   I   A   V   L   E   L   Q
                  C       C       G   G               G   C
529  CCC CTG GAC CGT CCA ACT TGG CAA GTA AGC AAT GCT GTT CAT CAG TCC
      P   L   D   R   P   T   W   Q   V   S   N   A   V   H   Q   S
          C                   C               C       C   C   G
577  TCT GCA TAC CAC GCC CCT CTG CAG CTG CAA TCG TCC ATT GCA GAA ACA
       S   A   Y   H   A   P   L   Q   L   Q   S   S   I   A   E   T
```

Figure 1B cont'd

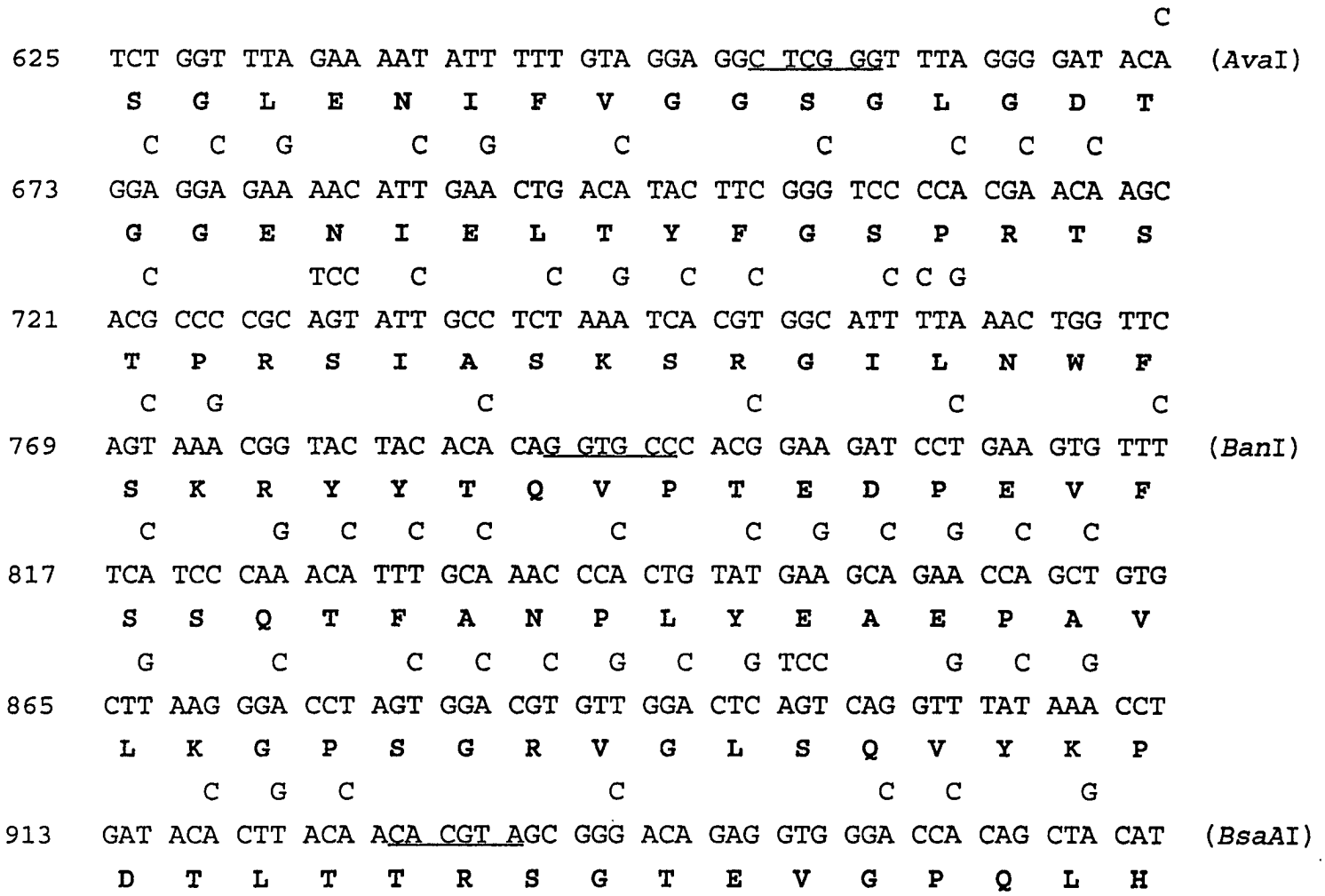

Figure 1B cont'd

```
       G          C C   TCC   C   C       G       G   G   T
961    GTC AGG TAC TCA TTG AGT ACT ATA CAT GAA GAT GTA GAA GCA ATC CCC
       V   R   Y   S   L   S   T   I   H   E   D   V   E   A   I   P
           C   G       G   C   C       C   G   C       G       C
1009   TAC ACA GTT GAT GAA AAT ACA CAG GGA CTT GCA TTC GTA CCC TTG CAT
       Y   T   V   D   E   N   T   Q   G   L   A   F   V   P   L   H
        G      G   C   C   C           C   G C C   C       C   C
1057   GAA GAG CAA GCA GGT TTT GAG GAG ATA GAA TTA GAT GAT TTT AGT GAG   (SacI)
       E   E   Q   A   G   F   E   E   I   E   L   D   D   F   S   E
        C     C C       G   C               C   C   C   C   G   C   C
1105   ACA CAT AGA CTG CTA CCT CAG AAC ACC TCT TCT ACA CCT GTT GGT AGT
       T   H   R   L   L   P   Q   N   T   S   S   T   P   V   G   S
        C   G   C           G   C   T   C       G   C   C   C   C
1153   GGT GTA CGA AGA AGC CTC ATT CCA ACT CGA GAA TTT AGT GCA ACA CGG
       G   V   R   R   S   L   I   P   T   Q   E   F   S   A   T   R
           C   C   G   G       C       C   C       C       C
1201   CCT ACA GGT GTT GTA ACC TAT GGC TCA CCT GAC ACT TAC TCT GCT AGC   (NheI)
       P   T   G   V   V   T   Y   G   S   P   D   T   Y   S   A   S
        C   G   C                               C   G   G       C
1249   CCA GTT ACT GAC CCT GAT TCT ACC TCT CCT AGT CTA GTT ATC GAT GAC
       P   V   T   D   P   D   S   T   S   P   S   L   V   I   D   D
```

Figure 1B cont'd

```
       C   C   C   C   C       C   C   C   C   C           G     C
1297  ACT ACT ACT ACA CCA ATC ATT ATA ATT GAT GGG CAC ACA GTT GAT TTG
       T   T   T   T   P   I   I   I   I   D   G   H   T   V   D   L
           C               C                   C   C         G   C   G
1345  TAC AGC AGT AAC TAC ACC TTG CAT CCC TCC TTG TTG AGG AAA CGA AAA
       Y   S   S   N   Y   T   L   H   P   S   L   L   R   K   R   K
        G   C   G
1393  AAA CGG AAA CAT GCC TAA
       K   R   K   H   A   -
```

Figure 1C

```
          T   A   G   A       A   T   A   G       G   T   A   A
1     ATG AGC AAG GGC GAG GAA CTG TTC ACT GGC GTG GTC CCA ATT CTC GTG
       M   S   K   G   E   E   L   F   T   G   V   V   P   I   L   V
         A       G                                           T   G   A
49    GAA CTG GAT GGC GAT GTG AAT GGG CAC AAA TTT TCT GTC AGC GGA GAG
       E   L   D   G   D   V   N   G   H   K   F   S   V   S   G   E
        G       G       A       T   G   A   A   A   A       T   A
97    GGT GAA GGT GAT GCC ACA TAC GGA AAG CTC ACC CTG AAA TTC ATC TGC
       G   E   G   D   A   T   Y   G   K   L   T   L   K   F   I   C
        A   A   G   A   A                   T       A   G   A   A   T
145   ACC ACT GGA AAG CTC CCT GTG CCA TGG CCA ACA CTG GTC ACT ACC TTC   (NcoI)
       T   T   G   K   L   P   V   P   W   P   T   L   V   T   T   F
      AG       G       A         AGT       T   T   T           A   A
193   TCT TAT GGC GTG CAG TGC TTT TCC AGA TAC CCA GAC CAT ATG AAG CAG
       S   Y   G   V   Q   C   F   S   R   Y   P   D   H   M   K   Q
           T       T   A   T   A               G           A   A
241   CAT GAC TTT TTC AAG AGC GCC ATG CCC GAG GGC TAT GTG CAG GAG AGA   (AvaI)
       H   D   F   F   K   S   A   M   P   E   G   Y   V   Q   E   R
        A   A       T           T       T   T   A   A A A   A       G
289   ACC ATC TTT TTC AAA GAT GAC GGG AAC TAC AAG ACC CGC GCT GAA GTC
       T   I   F   F   K   D   D   G   N   Y   K   T   R   A   E   V
```

Figure 1C cont'd

```
        A   T       G   T   A   A               A       C   A   G   A
337     AAG TTC GAA GGT GAC ACC CTG GTG AAT AGA ATC GAG CTG AAG GGC ATT   (SacI)
        K   F   E   G   D   T   L   V   N   R   I   E   L   K   G   I
        T       A   A       G   T   A   A   G   T   A   A       T   T
385     GAC TTT AAG GAG GAT GGA AAC ATT CTC GGC CAC AAG CTG GAA TAC AAC
        D   F   K   E   D   G   N   I   L   G   H   K   L   E   Y   N
            T AGT   T           T   A       A   T   A       A       G
433     TAT AAC TCC CAC AAT GTG TAC ATC ATG GCC GAC AAG CAA AAG AAT GGC
        Y   N   S   H   N   V   Y   I   M   A   D   K   Q   K   N   G
        A   A   G   T   T   A   A   T AGA CAT   A   A
481     ATC AAG GTC AAC TTC AAG ATC AGA CAC AAC ATT GAG GAT GGA TCC GTG   BamHI)
        I   K   V   N   F   K   I   R   H   N   I   E   D   G   S   V
        A   A   A   T               A   T   A   T   A   G   T   G
529     CAG CTG GCC GAC CAT TAT CAA CAG AAC ACT CCA ATC GGC GAC GGC CCT
        Q   L   A   D   H   Y   Q   Q   N   T   P   I   G   D   G   P
            A   A   T   T           T   A AGT   A   A AGT   A   A AG
577     GTG CTC CTC CCA GAC AAC CAT TAC CTG TCC ACC CAG TCT GCC CTG TCT
        V   L   L   P   D   N   H   Y   L   S   T   Q   S   A   L   S
                T   T       A       T   T       G   A   C
625     AAA GAT CCC AAC GAA AAG AGA GAC CAC ATG GTC CTG CTG GAG TTT GTG   (XhoI)
        K   D   P   N   E   K   R   D   H   M   V   L   L   E   F   V
```

Figure 1C cont'd

```
      A   A   A       A           G       T   A   A   T   A
673   ACC GCT GCT GGG ATC ACA CAT GGC ATG GAC GAG CTG TAC AAG TGA
      T   A   A   G   I   T   H   G   M   D   E   L   Y   K   -
```

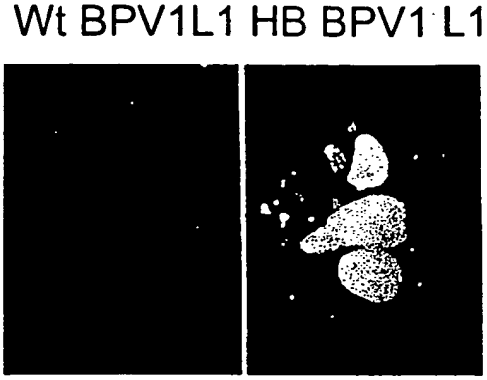

FIG. 2A

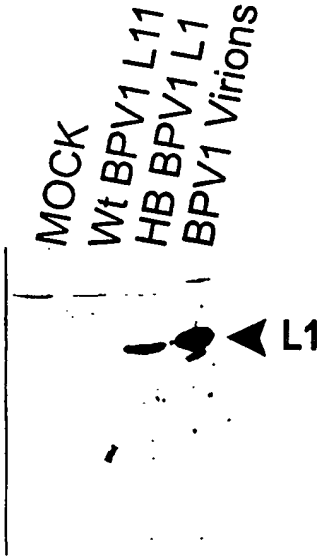

FIG. 2B

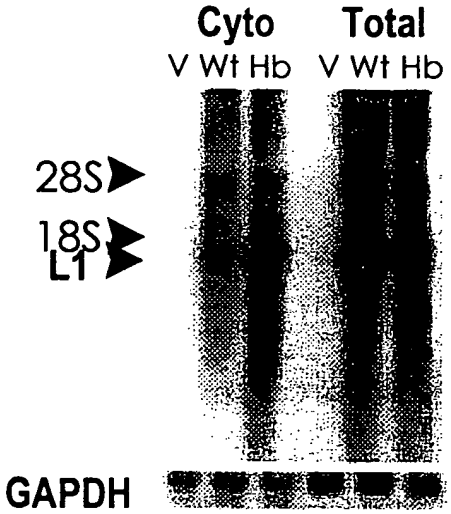

FIG. 2C

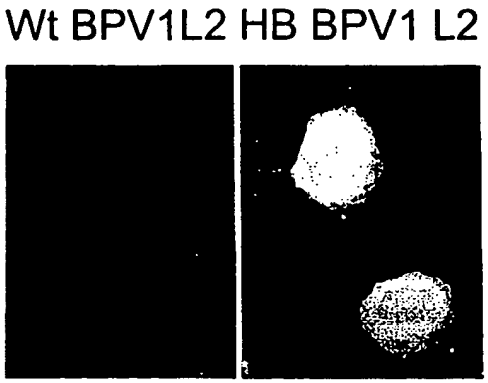

FIG. 3A

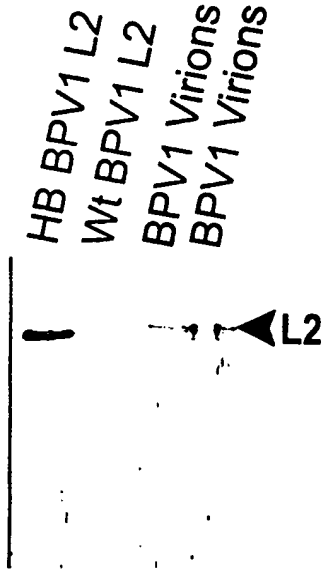

FIG. 3B

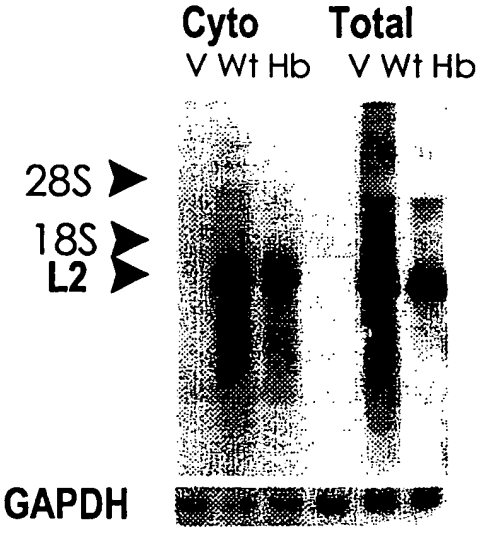

FIG. 3C

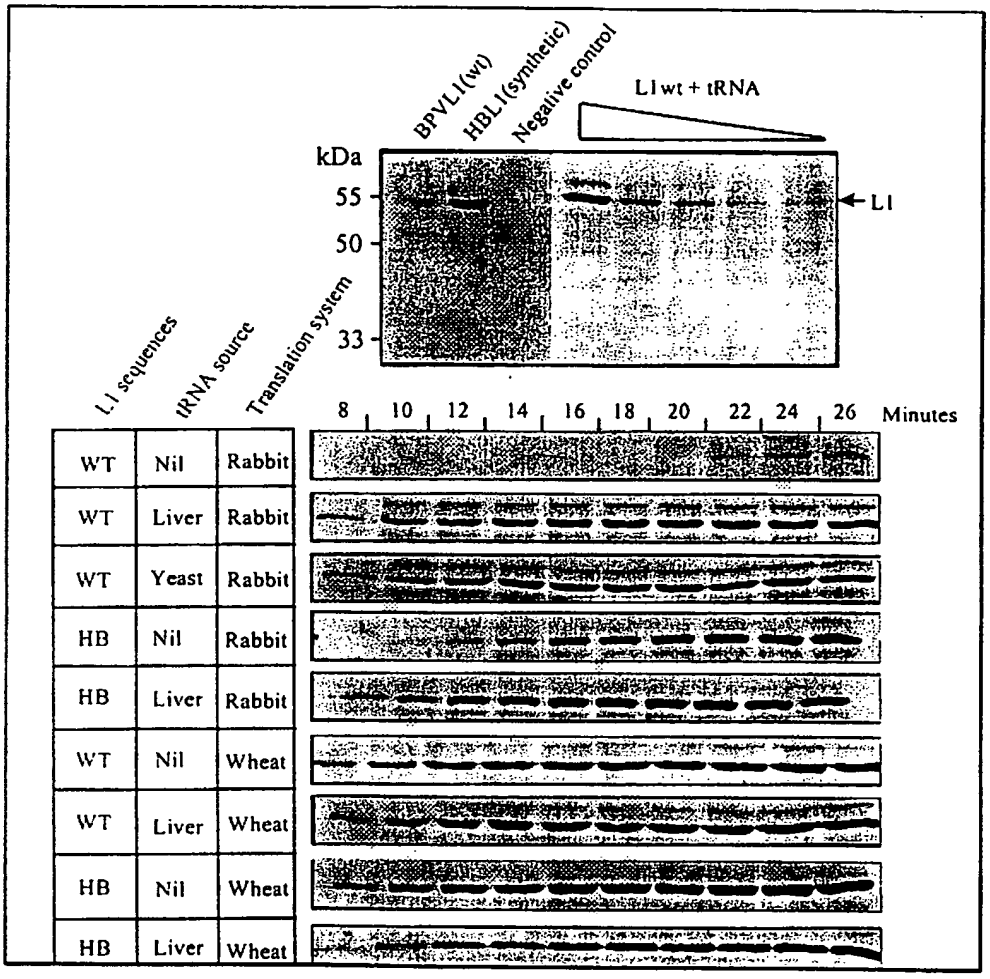
BPVL1(wt)
HBL1(synthetic)
Negative control
L1wt + tRNA
kDa
55
50
33
L1
L1 sequences
tRNA source
Translation system
8 10 12 14 16 18 20 22 24 26 Minutes
WT Nil Rabbit
WT Liver Rabbit
WT Yeast Rabbit
HB Nil Rabbit
HB Liver Rabbit
WT Nil Wheat
WT Liver Wheat
HB Nil Wheat
HB Liver Wheat

FIG. 4

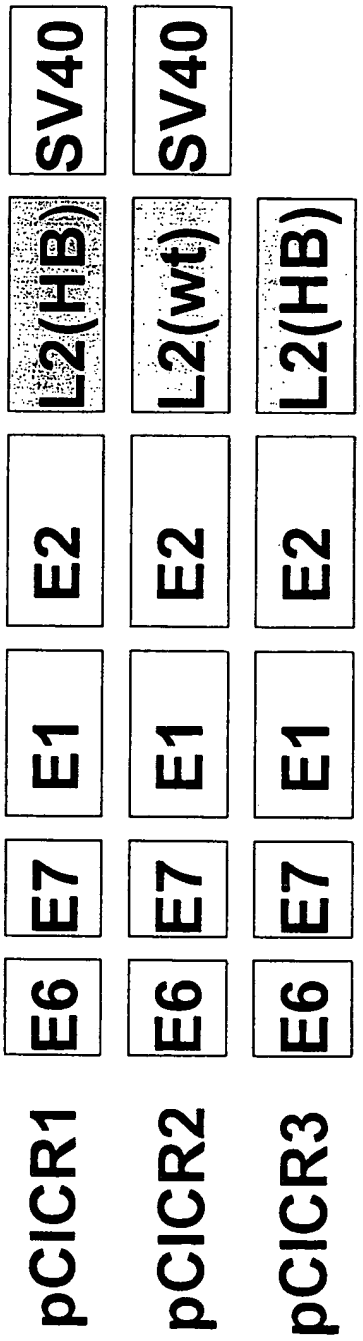
pCICR1
E6
E7
E1
E2
L2(HB)
SV40
pCICR2
E6
E7
E1
E2
L2(wt)
SV40
pCICR3
E6
E7
E1
E2
L2(HB)

FIG. 5A

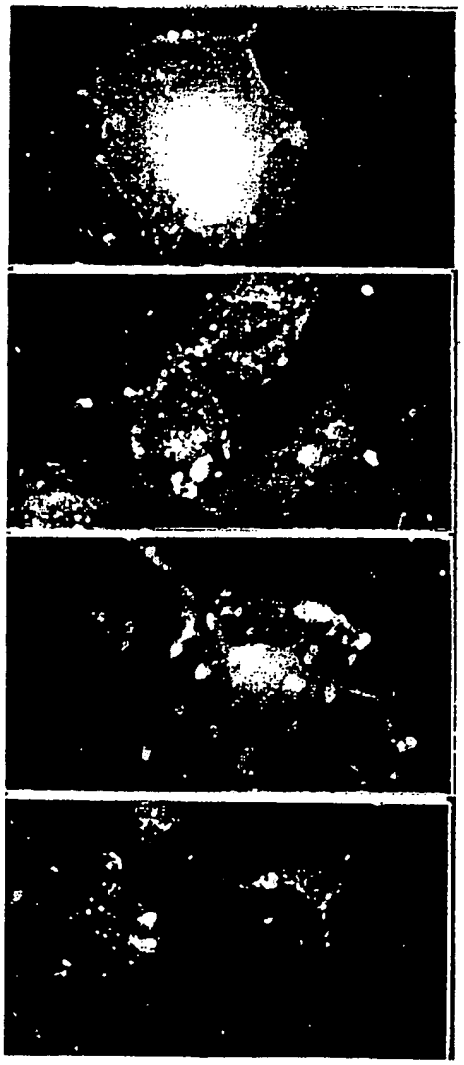
Mock
pCICR3
pCICR2
pCICR1

FIG. 5B

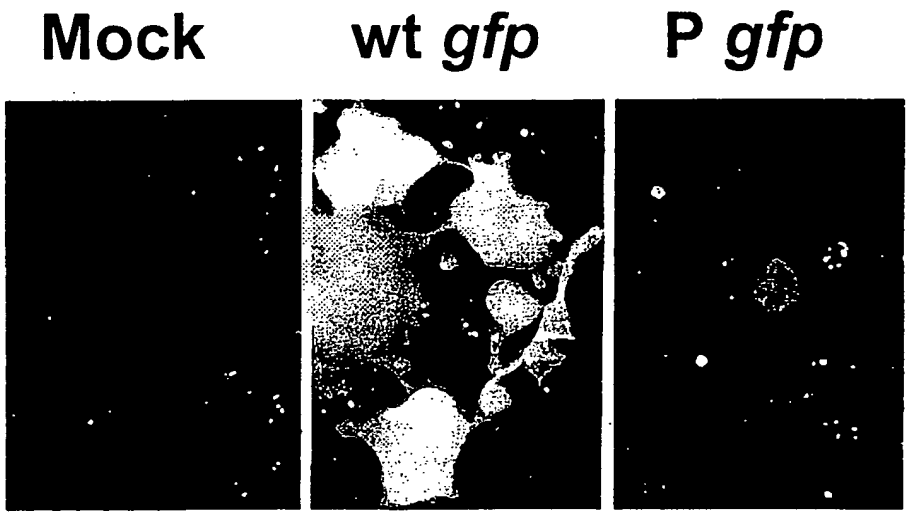

FIG. 6A

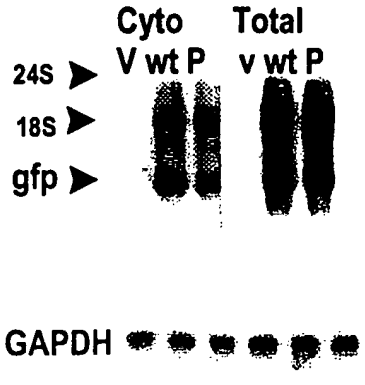

FIG. 6B

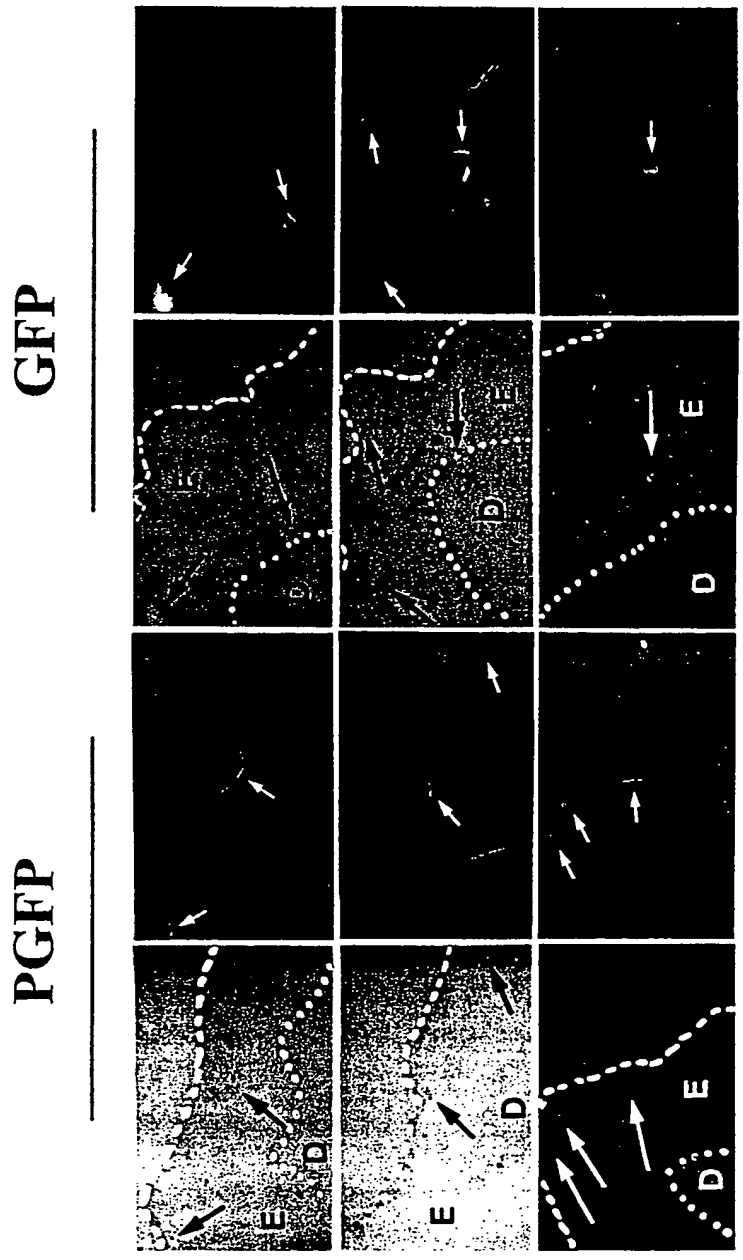

FIG. 7

SEQUENCE LISTING

<110> The University of Queensland

<120> NUCLEIC ACID SEQUENCE AND METHOD FOR SELECTIVELY EXPRESSING A PROTEIN IN A TARGET CELL OR TISSUE

<130> Selective Expression

<140> PCT/AU98/00530
<141> 1998-07-09

<150> PO7765
<151> 1997-07-09

<150> PO9467
<151> 1997-09-11

<160> 34

<170> PatentIn Ver. 2.0

<210> 1
<211> 1488
<212> DNA
<213> Bovine papillomavirus type 1

<220>
<221> CDS
<222> (1)..(1488)

<220>
<223> L1 open reading frame (wild-type)

<400> 1

```
atg gcg ttg tgg caa caa ggc cag aag ctg tat ctc cct cca acc cct   48
Met Ala Leu Trp Gln Gln Gly Gln Lys Leu Tyr Leu Pro Pro Thr Pro
  1               5                  10                  15

gta agc aag gtg ctt tgc agt gaa acc tat gtg caa aga aaa agc att   96
Val Ser Lys Val Leu Cys Ser Glu Thr Tyr Val Gln Arg Lys Ser Ile
             20                  25                  30

ttt tat cat gca gaa acg gag cgc ctg cta act ata gga cat cca tat   144
Phe Tyr His Ala Glu Thr Glu Arg Leu Leu Thr Ile Gly His Pro Tyr
         35                  40                  45

tac cca gtg tct atc ggg gcc aaa act gtt cct aag gtc tct gca aat   192
Tyr Pro Val Ser Ile Gly Ala Lys Thr Val Pro Lys Val Ser Ala Asn
     50                  55                  60

cag tat agg gta ttt aaa ata caa cta cct gat ccc aat caa ttt gca   240
Gln Tyr Arg Val Phe Lys Ile Gln Leu Pro Asp Pro Asn Gln Phe Ala
 65                  70                  75                  80
```

```
cta cct gac agg act gtt cac aac cca agt aaa gag cgg ctg gtg tgg   288
Leu Pro Asp Arg Thr Val His Asn Pro Ser Lys Glu Arg Leu Val Trp
                 85                  90                  95

cca gtc ata ggt gtg cag gtg tcc aga ggg cag cct ctt gga ggt act   336
Pro Val Ile Gly Val Gln Val Ser Arg Gly Gln Pro Leu Gly Gly Thr
            100                 105                 110

gta act ggg cac ccc act ttt aat gct ttg ctt gat gca gaa aat gtg   384
Val Thr Gly His Pro Thr Phe Asn Ala Leu Leu Asp Ala Glu Asn Val
        115                 120                 125

aat aga aaa gtc acc acc caa aca aca gat gac agg aaa caa aca ggc   432
Asn Arg Lys Val Thr Thr Gln Thr Thr Asp Asp Arg Lys Gln Thr Gly
    130                 135                 140

cta gat gct aag caa caa cag att ctg ttg cta ggc tgt acc cct gct   480
Leu Asp Ala Lys Gln Gln Gln Ile Leu Leu Leu Gly Cys Thr Pro Ala
145                 150                 155                 160

gaa ggg gaa tat tgg aca aca gcc cgt cca tgt gtt act gat cgt cta   528
Glu Gly Glu Tyr Trp Thr Thr Ala Arg Pro Cys Val Thr Asp Arg Leu
                165                 170                 175

gaa aat ggc gcc tgc cct cct ctt gaa tta aaa aac aag cac ata gaa   576
Glu Asn Gly Ala Cys Pro Pro Leu Glu Leu Lys Asn Lys His Ile Glu
            180                 185                 190

gat ggg gat atg atg gaa att ggg ttt ggt gca gcc aac ttc aaa gaa   624
Asp Gly Asp Met Met Glu Ile Gly Phe Gly Ala Ala Asn Phe Lys Glu
        195                 200                 205

att aat gca agt aaa tca gat cta cct ctt gac att caa aat gag atc   672
Ile Asn Ala Ser Lys Ser Asp Leu Pro Leu Asp Ile Gln Asn Glu Ile
    210                 215                 220

tgc ttg tac cca gac tac ctc aaa atg gct gag gac gct gct ggt aat   720
Cys Leu Tyr Pro Asp Tyr Leu Lys Met Ala Glu Asp Ala Ala Gly Asn
225                 230                 235                 240

agc atg ttc ttt ttt gca agg aaa gaa cag gtg tat gtt aga cac atc   768
Ser Met Phe Phe Phe Ala Arg Lys Glu Gln Val Tyr Val Arg His Ile
                245                 250                 255

tgg acc aga ggg ggc tcg gag aaa gaa gcc cct acc aca gat ttt tat   816
Trp Thr Arg Gly Gly Ser Glu Lys Glu Ala Pro Thr Thr Asp Phe Tyr
            260                 265                 270

tta aag aat aat aaa ggg gat gcc acc ctt aaa ata ccc agt gtg cat   864
Leu Lys Asn Asn Lys Gly Asp Ala Thr Leu Lys Ile Pro Ser Val His
        275                 280                 285

ttt ggt agt ccc agt ggc tca cta gtc tca act gat aat caa att ttt   912
Phe Gly Ser Pro Ser Gly Ser Leu Val Ser Thr Asp Asn Gln Ile Phe
    290                 295                 300
```

```
aat cgg ccc tac tgg cta ttc cgt gcc cag ggc atg aac aat gga att   960
Asn Arg Pro Tyr Trp Leu Phe Arg Ala Gln Gly Met Asn Asn Gly Ile
305                 310                 315                 320

gca tgg aat aat tta ttg ttt tta aca gtg ggg gac aat aca cgt ggt   1008
Ala Trp Asn Asn Leu Leu Phe Leu Thr Val Gly Asp Asn Thr Arg Gly
                325                 330                 335

act aat ctt acc ata agt gta gcc tca gat gga acc cca cta aca gag   1056
Thr Asn Leu Thr Ile Ser Val Ala Ser Asp Gly Thr Pro Leu Thr Glu
            340                 345                 350

tat gat agc tca aaa ttc aat gta tac cat aga cat atg gaa gaa tat   1104
Tyr Asp Ser Ser Lys Phe Asn Val Tyr His Arg His Met Glu Glu Tyr
        355                 360                 365

aag cta gcc ttt ata tta gag cta tgc tct gtg gaa atc aca gct caa   1152
Lys Leu Ala Phe Ile Leu Glu Leu Cys Ser Val Glu Ile Thr Ala Gln
    370                 375                 380

act gtg tca cat ctg caa gga ctt atg ccc tct gtg ctt gaa aat tgg   1200
Thr Val Ser His Leu Gln Gly Leu Met Pro Ser Val Leu Glu Asn Trp
385                 390                 395                 400

gaa ata ggt gtg cag cct cct acc tca tcg ata tta gag gac acc tat   1248
Glu Ile Gly Val Gln Pro Pro Thr Ser Ser Ile Leu Glu Asp Thr Tyr
                405                 410                 415

cgc tat ata gag tct cct gca act aaa tgt gca agc aat gta att cct   1296
Arg Tyr Ile Glu Ser Pro Ala Thr Lys Cys Ala Ser Asn Val Ile Pro
            420                 425                 430

gca aaa gaa gac cct tat gca ggg ttt aag ttt tgg aac ata gat ctt   1344
Ala Lys Glu Asp Pro Tyr Ala Gly Phe Lys Phe Trp Asn Ile Asp Leu
        435                 440                 445

aaa gaa aag ctt tct ttg gac tta gat caa ttt ccc ttg gga aga aga   1392
Lys Glu Lys Leu Ser Leu Asp Leu Asp Gln Phe Pro Leu Gly Arg Arg
    450                 455                 460

ttt tta gca cag caa ggg gca gga tgt tca act gtg aga aaa cga aga   1440
Phe Leu Ala Gln Gln Gly Ala Gly Cys Ser Thr Val Arg Lys Arg Arg
465                 470                 475                 480

att agc caa aaa act tcc agt aag cct gca aaa aaa aaa aaa aaa taa   1488
Ile Ser Gln Lys Thr Ser Ser Lys Pro Ala Lys Lys Lys Lys Lys
                485                 490                 495
```

<210> 2
<211> 495
<212> PRT
<213> Bovine papillomavirus type 1

<400> 2

```
Met Ala Leu Trp Gln Gln Gly Gln Lys Leu Tyr Leu Pro Pro Thr Pro
  1               5                  10                  15
```

```
Val Ser Lys Val Leu Cys Ser Glu Thr Tyr Val Gln Arg Lys Ser Ile
            20                  25                  30

Phe Tyr His Ala Glu Thr Glu Arg Leu Leu Thr Ile Gly His Pro Tyr
        35                  40                  45

Tyr Pro Val Ser Ile Gly Ala Lys Thr Val Pro Lys Val Ser Ala Asn
    50                  55                  60

Gln Tyr Arg Val Phe Lys Ile Gln Leu Pro Asp Pro Asn Gln Phe Ala
65                  70                  75                  80

Leu Pro Asp Arg Thr Val His Asn Pro Ser Lys Glu Arg Leu Val Trp
                85                  90                  95

Pro Val Ile Gly Val Gln Val Ser Arg Gly Gln Pro Leu Gly Gly Thr
            100                 105                 110

Val Thr Gly His Pro Thr Phe Asn Ala Leu Leu Asp Ala Glu Asn Val
        115                 120                 125

Asn Arg Lys Val Thr Thr Gln Thr Thr Asp Asp Arg Lys Gln Thr Gly
    130                 135                 140

Leu Asp Ala Lys Gln Gln Gln Ile Leu Leu Leu Gly Cys Thr Pro Ala
145                 150                 155                 160

Glu Gly Glu Tyr Trp Thr Thr Ala Arg Pro Cys Val Thr Asp Arg Leu
                165                 170                 175

Glu Asn Gly Ala Cys Pro Pro Leu Glu Leu Lys Asn Lys His Ile Glu
            180                 185                 190

Asp Gly Asp Met Met Glu Ile Gly Phe Gly Ala Ala Asn Phe Lys Glu
        195                 200                 205

Ile Asn Ala Ser Lys Ser Asp Leu Pro Leu Asp Ile Gln Asn Glu Ile
    210                 215                 220

Cys Leu Tyr Pro Asp Tyr Leu Lys Met Ala Glu Asp Ala Ala Gly Asn
225                 230                 235                 240

Ser Met Phe Phe Phe Ala Arg Lys Glu Gln Val Tyr Val Arg His Ile
                245                 250                 255

Trp Thr Arg Gly Gly Ser Glu Lys Glu Ala Pro Thr Thr Asp Phe Tyr
            260                 265                 270

Leu Lys Asn Asn Lys Gly Asp Ala Thr Leu Lys Ile Pro Ser Val His
        275                 280                 285

Phe Gly Ser Pro Ser Gly Ser Leu Val Ser Thr Asp Asn Gln Ile Phe
    290                 295                 300

Asn Arg Pro Tyr Trp Leu Phe Arg Ala Gln Gly Met Asn Asn Gly Ile
305                 310                 315                 320
```

```
Ala Trp Asn Asn Leu Leu Phe Leu Thr Val Gly Asp Asn Thr Arg Gly
                325                 330                 335

Thr Asn Leu Thr Ile Ser Val Ala Ser Asp Gly Thr Pro Leu Thr Glu
            340                 345                 350

Tyr Asp Ser Ser Lys Phe Asn Val Tyr His Arg His Met Glu Glu Tyr
        355                 360                 365

Lys Leu Ala Phe Ile Leu Glu Leu Cys Ser Val Glu Ile Thr Ala Gln
    370                 375                 380

Thr Val Ser His Leu Gln Gly Leu Met Pro Ser Val Leu Glu Asn Trp
385                 390                 395                 400

Glu Ile Gly Val Gln Pro Pro Thr Ser Ser Ile Leu Glu Asp Thr Tyr
                405                 410                 415

Arg Tyr Ile Glu Ser Pro Ala Thr Lys Cys Ala Ser Asn Val Ile Pro
            420                 425                 430

Ala Lys Glu Asp Pro Tyr Ala Gly Phe Lys Phe Trp Asn Ile Asp Leu
        435                 440                 445

Lys Glu Lys Leu Ser Leu Asp Leu Asp Gln Phe Pro Leu Gly Arg Arg
    450                 455                 460

Phe Leu Ala Gln Gln Gly Ala Gly Cys Ser Thr Val Arg Lys Arg Arg
465                 470                 475                 480

Ile Ser Gln Lys Thr Ser Ser Lys Pro Ala Lys Lys Lys Lys Lys
                485                 490                 495
```

<210> 3
<211> 1488
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(1488)

<220>
<223> Description of Artificial Sequence: Bovine papillomavirus type 1 L1 open reading frame (humanized)

<220>
<223> Wild-type codons replaced with synonymous codons used at relatively high frequency by human genes

<400> 3

```
atg gcc ctg tgg cag cag ggc cag aag ctg tac ctg ccc cct acc ccc   48
Met Ala Leu Trp Gln Gln Gly Gln Lys Leu Tyr Leu Pro Pro Thr Pro
  1               5                  10                  15
```

```
gtg agc aag gtg ctt tgc agt gaa acc tat gtg caa aga aaa agc att   96
Val Ser Lys Val Leu Cys Ser Glu Thr Tyr Val Gln Arg Lys Ser Ile
             20                  25                  30

ttt tat cat gca gaa acg gag cgc ctg ctg acc atc gga cac ccc tat   144
Phe Tyr His Ala Glu Thr Glu Arg Leu Leu Thr Ile Gly His Pro Tyr
         35                  40                  45

tac ccc gtg tcc atc ggg gcc aag act gtg cct aag gtg tcc gcc aat   192
Tyr Pro Val Ser Ile Gly Ala Lys Thr Val Pro Lys Val Ser Ala Asn
     50                  55                  60

cag tat agg gtg ttc aaa atc caa ctg cct gat ccc aat caa ttt gca   240
Gln Tyr Arg Val Phe Lys Ile Gln Leu Pro Asp Pro Asn Gln Phe Ala
 65                  70                  75                  80

ctg cct gac agg acc gtg cac aac ccc agc aaa gag cgg ctg gtg tgg   288
Leu Pro Asp Arg Thr Val His Asn Pro Ser Lys Glu Arg Leu Val Trp
                 85                  90                  95

cca gtg atc ggc gtg cag gtg tcc aga ggc cag cct ctg ggc ggc acc   336
Pro Val Ile Gly Val Gln Val Ser Arg Gly Gln Pro Leu Gly Gly Thr
            100                 105                 110

gtg act ggg cac ccc act ttt aat gct ttg ctt gat gca gaa aat gtg   384
Val Thr Gly His Pro Thr Phe Asn Ala Leu Leu Asp Ala Glu Asn Val
        115                 120                 125

aat aga aaa gtc acc acc cag acc acc gac gac agg aaa cag aca ggc   432
Asn Arg Lys Val Thr Thr Gln Thr Thr Asp Asp Arg Lys Gln Thr Gly
    130                 135                 140

ctg gat gcc aag cag cag cag atc ctg ctg ctg ggc tgt acc cct gct   480
Leu Asp Ala Lys Gln Gln Gln Ile Leu Leu Leu Gly Cys Thr Pro Ala
145                 150                 155                 160

gaa ggg gaa tat tgg aca aca gcc cgt cca tgt gtg acc gac cgt cta   528
Glu Gly Glu Tyr Trp Thr Thr Ala Arg Pro Cys Val Thr Asp Arg Leu
                165                 170                 175

gaa aac ggc gcc tgc cct cct ctg gag ctg aaa aac aag cac atc gaa   576
Glu Asn Gly Ala Cys Pro Pro Leu Glu Leu Lys Asn Lys His Ile Glu
            180                 185                 190

gat ggg gat atg atg gaa att ggg ttt ggt gca gcc aac ttc aaa gaa   624
Asp Gly Asp Met Met Glu Ile Gly Phe Gly Ala Ala Asn Phe Lys Glu
        195                 200                 205

att aat gca agt aaa tca gat cta cct ctg gac atc caa aat gag atc   672
Ile Asn Ala Ser Lys Ser Asp Leu Pro Leu Asp Ile Gln Asn Glu Ile
    210                 215                 220

tgc ctg tac ccc gac tac ctg aaa atg gct gag gac gcc gcc ggc aac   720
Cys Leu Tyr Pro Asp Tyr Leu Lys Met Ala Glu Asp Ala Ala Gly Asn
225                 230                 235                 240
```

```
agc atg ttc ttc ttc gcc agg aag gag cag gtg tac gtg aga cac atc   768
Ser Met Phe Phe Phe Ala Arg Lys Glu Gln Val Tyr Val Arg His Ile
                245                 250                 255

tgg acc aga ggc ggc tcc gag aaa gaa gcc cct acc aca gat ttt tat   816
Trp Thr Arg Gly Gly Ser Glu Lys Glu Ala Pro Thr Thr Asp Phe Tyr
            260                 265                 270

ttg aag aac aac aag ggc gac gcc acc ctg aag atc ccc agc gtg cac   864
Leu Lys Asn Asn Lys Gly Asp Ala Thr Leu Lys Ile Pro Ser Val His
        275                 280                 285

ttc ggc agc ccc agc ggc tca cta gtg tcc acc gac aac cag atc ttc   912
Phe Gly Ser Pro Ser Gly Ser Leu Val Ser Thr Asp Asn Gln Ile Phe
    290                 295                 300

aac cgg ccc tac tgg ctg ttc cgc gcc cag ggc atg aac aat gga att   960
Asn Arg Pro Tyr Trp Leu Phe Arg Ala Gln Gly Met Asn Asn Gly Ile
305                 310                 315                 320

gcc tgg aac aac ctg ctg ttc ctg acc gtg ggc gac aac aca cgt ggc   1008
Ala Trp Asn Asn Leu Leu Phe Leu Thr Val Gly Asp Asn Thr Arg Gly
                325                 330                 335

acc aac ctg acc atc agc gtg gcc tcc gat gga acc cca ctg acc gag   1056
Thr Asn Leu Thr Ile Ser Val Ala Ser Asp Gly Thr Pro Leu Thr Glu
            340                 345                 350

tat gat agc tcg aaa ttc aac gtg tac cac aga cac atg gag gag tat   1104
Tyr Asp Ser Ser Lys Phe Asn Val Tyr His Arg His Met Glu Glu Tyr
        355                 360                 365

aag cta gcc ttc atc ctg gag ctg tgc tcc gtg gag atc acc gcc cag   1152
Lys Leu Ala Phe Ile Leu Glu Leu Cys Ser Val Glu Ile Thr Ala Gln
    370                 375                 380

acc gtg tcc cat ctg caa gga ctg atg ccc tcc gtg ctg gag aat tgg   1200
Thr Val Ser His Leu Gln Gly Leu Met Pro Ser Val Leu Glu Asn Trp
385                 390                 395                 400

gag atc ggc gtg cag ccc ccc acc tca tcg atc ttg gag gac acc tac   1248
Glu Ile Gly Val Gln Pro Pro Thr Ser Ser Ile Leu Glu Asp Thr Tyr
                405                 410                 415

cgc tac atc gag tcc ccc gcc acc aag tgt gcc agc aac gtg att cct   1296
Arg Tyr Ile Glu Ser Pro Ala Thr Lys Cys Ala Ser Asn Val Ile Pro
            420                 425                 430

gca aaa gaa gac cct tat gca ggg ttt aag ttc tgg aac atc gac ctg   1344
Ala Lys Glu Asp Pro Tyr Ala Gly Phe Lys Phe Trp Asn Ile Asp Leu
        435                 440                 445

aag gag aag ctg tct ctg gac ctg gat cag ttc ccc ttg ggc aga aga   1392
Lys Glu Lys Leu Ser Leu Asp Leu Asp Gln Phe Pro Leu Gly Arg Arg
    450                 455                 460
```

```
ttt ctg gcc cag cag ggg gcc ggc tgt tcc acc gtg aga aaa cgc agg   1440
Phe Leu Ala Gln Gln Gly Ala Gly Cys Ser Thr Val Arg Lys Arg Arg
465                 470                 475                 480

atc agc cag aag acc tcc agc aag ccc gcc aag aag aag aaa aag taa   1488
Ile Ser Gln Lys Thr Ser Ser Lys Pro Ala Lys Lys Lys Lys Lys
                485                 490                 495
```

<210> 4
<211> 495
<212> PRT
<213> Artificial Sequence

<400> 4

```
Met Ala Leu Trp Gln Gln Gly Gln Lys Leu Tyr Leu Pro Pro Thr Pro
  1               5                  10                  15

Val Ser Lys Val Leu Cys Ser Glu Thr Tyr Val Gln Arg Lys Ser Ile
             20                  25                  30

Phe Tyr His Ala Glu Thr Glu Arg Leu Leu Thr Ile Gly His Pro Tyr
         35                  40                  45

Tyr Pro Val Ser Ile Gly Ala Lys Thr Val Pro Lys Val Ser Ala Asn
     50                  55                  60

Gln Tyr Arg Val Phe Lys Ile Gln Leu Pro Asp Pro Asn Gln Phe Ala
 65                  70                  75                  80

Leu Pro Asp Arg Thr Val His Asn Pro Ser Lys Glu Arg Leu Val Trp
                 85                  90                  95

Pro Val Ile Gly Val Gln Val Ser Arg Gly Gln Pro Leu Gly Gly Thr
            100                 105                 110

Val Thr Gly His Pro Thr Phe Asn Ala Leu Leu Asp Ala Glu Asn Val
        115                 120                 125

Asn Arg Lys Val Thr Thr Gln Thr Thr Asp Asp Arg Lys Gln Thr Gly
    130                 135                 140

Leu Asp Ala Lys Gln Gln Gln Ile Leu Leu Leu Gly Cys Thr Pro Ala
145                 150                 155                 160

Glu Gly Glu Tyr Trp Thr Thr Ala Arg Pro Cys Val Thr Asp Arg Leu
                165                 170                 175

Glu Asn Gly Ala Cys Pro Pro Leu Glu Leu Lys Asn Lys His Ile Glu
            180                 185                 190

Asp Gly Asp Met Met Glu Ile Gly Phe Gly Ala Ala Asn Phe Lys Glu
        195                 200                 205

Ile Asn Ala Ser Lys Ser Asp Leu Pro Leu Asp Ile Gln Asn Glu Ile
    210                 215                 220
```

```
Cys Leu Tyr Pro Asp Tyr Leu Lys Met Ala Glu Asp Ala Ala Gly Asn
225                 230                 235                 240

Ser Met Phe Phe Phe Ala Arg Lys Glu Gln Val Tyr Val Arg His Ile
                245                 250                 255

Trp Thr Arg Gly Gly Ser Glu Lys Glu Ala Pro Thr Thr Asp Phe Tyr
            260                 265                 270

Leu Lys Asn Asn Lys Gly Asp Ala Thr Leu Lys Ile Pro Ser Val His
        275                 280                 285

Phe Gly Ser Pro Ser Gly Ser Leu Val Ser Thr Asp Asn Gln Ile Phe
    290                 295                 300

Asn Arg Pro Tyr Trp Leu Phe Arg Ala Gln Gly Met Asn Asn Gly Ile
305                 310                 315                 320

Ala Trp Asn Asn Leu Leu Phe Leu Thr Val Gly Asp Asn Thr Arg Gly
                325                 330                 335

Thr Asn Leu Thr Ile Ser Val Ala Ser Asp Gly Thr Pro Leu Thr Glu
            340                 345                 350

Tyr Asp Ser Ser Lys Phe Asn Val Tyr His Arg His Met Glu Glu Tyr
        355                 360                 365

Lys Leu Ala Phe Ile Leu Glu Leu Cys Ser Val Glu Ile Thr Ala Gln
    370                 375                 380

Thr Val Ser His Leu Gln Gly Leu Met Pro Ser Val Leu Glu Asn Trp
385                 390                 395                 400

Glu Ile Gly Val Gln Pro Pro Thr Ser Ser Ile Leu Glu Asp Thr Tyr
                405                 410                 415

Arg Tyr Ile Glu Ser Pro Ala Thr Lys Cys Ala Ser Asn Val Ile Pro
            420                 425                 430

Ala Lys Glu Asp Pro Tyr Ala Gly Phe Lys Phe Trp Asn Ile Asp Leu
        435                 440                 445

Lys Glu Lys Leu Ser Leu Asp Leu Asp Gln Phe Pro Leu Gly Arg Arg
    450                 455                 460

Phe Leu Ala Gln Gln Gly Ala Gly Cys Ser Thr Val Arg Lys Arg Arg
465                 470                 475                 480

Ile Ser Gln Lys Thr Ser Ser Lys Pro Ala Lys Lys Lys Lys Lys
                485                 490                 495
```

<210> 5
<211> 1410
<212> DNA
<213> Bovine papillomavirus type 1

<220>
<221> CDS
<222> (1)..(1410)

<220>
<223> L2 open reading frame (wild-type)

<400> 5

```
atg agt gca cga aaa aga gta aaa cgt gcc agt gcc tat gac ctg tac   48
Met Ser Ala Arg Lys Arg Val Lys Arg Ala Ser Ala Tyr Asp Leu Tyr
  1               5                  10                  15

agg acc tgc aag caa gcg ggc aca tgt cca cca gat gtg ata cga aag   96
Arg Thr Cys Lys Gln Ala Gly Thr Cys Pro Pro Asp Val Ile Arg Lys
             20                  25                  30

gta gaa gga gat act ata gca gat aaa att ttg aaa ttt ggg ggt ctt   144
Val Glu Gly Asp Thr Ile Ala Asp Lys Ile Leu Lys Phe Gly Gly Leu
         35                  40                  45

gca atc tac tta gga ggg cta gga ata gga aca tgg tct act gga agg   192
Ala Ile Tyr Leu Gly Gly Leu Gly Ile Gly Thr Trp Ser Thr Gly Arg
     50                  55                  60

gtg gcc gca ggt gga tca cca agg tac aca cca ctc cga aca gca ggg   240
Val Ala Ala Gly Gly Ser Pro Arg Tyr Thr Pro Leu Arg Thr Ala Gly
 65                  70                  75                  80

tcc aca tca tcg ctt gca tca ata gga tcc aga gct gta aca gca ggg   288
Ser Thr Ser Ser Leu Ala Ser Ile Gly Ser Arg Ala Val Thr Ala Gly
                 85                  90                  95

acc cgc ccc agt ata ggt gcg ggc att cct tta gac acc ctt gaa act   336
Thr Arg Pro Ser Ile Gly Ala Gly Ile Pro Leu Asp Thr Leu Glu Thr
            100                 105                 110

ctt ggg gcc ttg cgt cca ggg gtg tat gag gac act gtg cta cca gag   384
Leu Gly Ala Leu Arg Pro Gly Val Tyr Glu Asp Thr Val Leu Pro Glu
        115                 120                 125

gcc cct gca ata gtc act cct gat gct gtt cct gca gat tca ggg ctt   432
Ala Pro Ala Ile Val Thr Pro Asp Ala Val Pro Ala Asp Ser Gly Leu
    130                 135                 140

gat gcc ctg tcc ata ggt aca gac tcg tcc acg gag acc ctc att act   480
Asp Ala Leu Ser Ile Gly Thr Asp Ser Ser Thr Glu Thr Leu Ile Thr
145                 150                 155                 160

ctg cta gag cct gag ggt ccc gag gac ata gcg gtt ctt gag ctg caa   528
Leu Leu Glu Pro Glu Gly Pro Glu Asp Ile Ala Val Leu Glu Leu Gln
                165                 170                 175

ccc ctg gac cgt cca act tgg caa gta agc aat gct gtt cat cag tcc   576
Pro Leu Asp Arg Pro Thr Trp Gln Val Ser Asn Ala Val His Gln Ser
            180                 185                 190
```

```
tct gca tac cac gcc cct ctg cag ctg caa tcg tcc att gca gaa aca   624
Ser Ala Tyr His Ala Pro Leu Gln Leu Gln Ser Ser Ile Ala Glu Thr
        195                 200                 205

tct ggt tta gaa aat att ttt gta gga ggc tcg ggt tta ggg gat aca   672
Ser Gly Leu Glu Asn Ile Phe Val Gly Gly Ser Gly Leu Gly Asp Thr
    210                 215                 220

gga gga gaa aac att gaa ctg aca tac ttc ggg tcc cca cga aca agc   720
Gly Gly Glu Asn Ile Glu Leu Thr Tyr Phe Gly Ser Pro Arg Thr Ser
225                 230                 235                 240

acg ccc cgc agt att gcc tct aaa tca cgt ggc att tta aac tgg ttc   768
Thr Pro Arg Ser Ile Ala Ser Lys Ser Arg Gly Ile Leu Asn Trp Phe
                245                 250                 255

agt aaa cgg tac tac aca cag gtg ccc acg gaa gat cct gaa gtg ttt   816
Ser Lys Arg Tyr Tyr Thr Gln Val Pro Thr Glu Asp Pro Glu Val Phe
            260                 265                 270

tca tcc caa aca ttt gca aac cca ctg tat gaa gca gaa cca gct gtg   864
Ser Ser Gln Thr Phe Ala Asn Pro Leu Tyr Glu Ala Glu Pro Ala Val
        275                 280                 285

ctt aag gga cct agt gga cgt gtt gga ctc agt cag gtt tat aaa cct   912
Leu Lys Gly Pro Ser Gly Arg Val Gly Leu Ser Gln Val Tyr Lys Pro
    290                 295                 300

gat aca ctt aca aca cgt agc ggg aca gag gtg gga cca cag cta cat   960
Asp Thr Leu Thr Thr Arg Ser Gly Thr Glu Val Gly Pro Gln Leu His
305                 310                 315                 320

gtc agg tac tca ttg agt act ata cat gaa gat gta gaa gca atc ccc   1008
Val Arg Tyr Ser Leu Ser Thr Ile His Glu Asp Val Glu Ala Ile Pro
                325                 330                 335

tac aca gtt gat gaa aat aca cag gga ctt gca ttc gta ccc ttg cat   1056
Tyr Thr Val Asp Glu Asn Thr Gln Gly Leu Ala Phe Val Pro Leu His
            340                 345                 350

gaa gag caa gca ggt ttt gag gag ata gaa tta gat gat ttt agt gag   1104
Glu Glu Gln Ala Gly Phe Glu Glu Ile Glu Leu Asp Asp Phe Ser Glu
        355                 360                 365

aca cat aga ctg cta cct cag aac acc tct tct aca cct gtt ggt agt   1152
Thr His Arg Leu Leu Pro Gln Asn Thr Ser Ser Thr Pro Val Gly Ser
    370                 375                 380

ggt gta cga aga agc ctc att cca act cga gaa ttt agt gca aca cgg   1200
Gly Val Arg Arg Ser Leu Ile Pro Thr Arg Glu Phe Ser Ala Thr Arg
385                 390                 395                 400

cct aca ggt gtt gta acc tat ggc tca cct gac act tac tct gct agc   1248
Pro Thr Gly Val Val Thr Tyr Gly Ser Pro Asp Thr Tyr Ser Ala Ser
                405                 410                 415
```

```
cca gtt act gac cct gat tct acc tct cct agt cta gtt atc gat gac   1296
Pro Val Thr Asp Pro Asp Ser Thr Ser Pro Ser Leu Val Ile Asp Asp
            420                 425                 430

act act act aca cca atc att ata att gat ggg cac aca gtt gat ttg   1344
Thr Thr Thr Thr Pro Ile Ile Ile Ile Asp Gly His Thr Val Asp Leu
        435                 440                 445

tac agc agt aac tac acc ttg cat ccc tcc ttg ttg agg aaa cga aaa   1392
Tyr Ser Ser Asn Tyr Thr Leu His Pro Ser Leu Leu Arg Lys Arg Lys
    450                 455                 460

aaa cgg aaa cat gcc taa                                          1410
Lys Arg Lys His Ala
465                 470
```

<210> 6
<211> 469
<212> PRT
<213> Bovine papillomavirus type 1

<400> 6

```
Met Ser Ala Arg Lys Arg Val Lys Arg Ala Ser Ala Tyr Asp Leu Tyr
  1               5                  10                  15

Arg Thr Cys Lys Gln Ala Gly Thr Cys Pro Pro Asp Val Ile Arg Lys
             20                  25                  30

Val Glu Gly Asp Thr Ile Ala Asp Lys Ile Leu Lys Phe Gly Gly Leu
         35                  40                  45

Ala Ile Tyr Leu Gly Gly Leu Gly Ile Gly Thr Trp Ser Thr Gly Arg
     50                  55                  60

Val Ala Ala Gly Gly Ser Pro Arg Tyr Thr Pro Leu Arg Thr Ala Gly
 65                  70                  75                  80

Ser Thr Ser Ser Leu Ala Ser Ile Gly Ser Arg Ala Val Thr Ala Gly
                 85                  90                  95

Thr Arg Pro Ser Ile Gly Ala Gly Ile Pro Leu Asp Thr Leu Glu Thr
            100                 105                 110

Leu Gly Ala Leu Arg Pro Gly Val Tyr Glu Asp Thr Val Leu Pro Glu
        115                 120                 125

Ala Pro Ala Ile Val Thr Pro Asp Ala Val Pro Ala Asp Ser Gly Leu
    130                 135                 140

Asp Ala Leu Ser Ile Gly Thr Asp Ser Ser Thr Glu Thr Leu Ile Thr
145                 150                 155                 160

Leu Leu Glu Pro Glu Gly Pro Glu Asp Ile Ala Val Leu Glu Leu Gln
                165                 170                 175
```

```
Pro Leu Asp Arg Pro Thr Trp Gln Val Ser Asn Ala Val His Gln Ser
            180                 185                 190

Ser Ala Tyr His Ala Pro Leu Gln Leu Gln Ser Ser Ile Ala Glu Thr
        195                 200                 205

Ser Gly Leu Glu Asn Ile Phe Val Gly Gly Ser Gly Leu Gly Asp Thr
    210                 215                 220

Gly Gly Glu Asn Ile Glu Leu Thr Tyr Phe Gly Ser Pro Arg Thr Ser
225                 230                 235                 240

Thr Pro Arg Ser Ile Ala Ser Lys Ser Arg Gly Ile Leu Asn Trp Phe
                245                 250                 255

Ser Lys Arg Tyr Tyr Thr Gln Val Pro Thr Glu Asp Pro Glu Val Phe
            260                 265                 270

Ser Ser Gln Thr Phe Ala Asn Pro Leu Tyr Glu Ala Glu Pro Ala Val
        275                 280                 285

Leu Lys Gly Pro Ser Gly Arg Val Gly Leu Ser Gln Val Tyr Lys Pro
    290                 295                 300

Asp Thr Leu Thr Thr Arg Ser Gly Thr Glu Val Gly Pro Gln Leu His
305                 310                 315                 320

Val Arg Tyr Ser Leu Ser Thr Ile His Glu Asp Val Glu Ala Ile Pro
                325                 330                 335

Tyr Thr Val Asp Glu Asn Thr Gln Gly Leu Ala Phe Val Pro Leu His
            340                 345                 350

Glu Glu Gln Ala Gly Phe Glu Glu Ile Glu Leu Asp Asp Phe Ser Glu
        355                 360                 365

Thr His Arg Leu Leu Pro Gln Asn Thr Ser Ser Thr Pro Val Gly Ser
    370                 375                 380

Gly Val Arg Arg Ser Leu Ile Pro Thr Arg Glu Phe Ser Ala Thr Arg
385                 390                 395                 400

Pro Thr Gly Val Val Thr Tyr Gly Ser Pro Asp Thr Tyr Ser Ala Ser
                405                 410                 415

Pro Val Thr Asp Pro Asp Ser Thr Ser Pro Ser Leu Val Ile Asp Asp
            420                 425                 430

Thr Thr Thr Thr Pro Ile Ile Ile Ile Asp Gly His Thr Val Asp Leu
        435                 440                 445

Tyr Ser Ser Asn Tyr Thr Leu His Pro Ser Leu Leu Arg Lys Arg Lys
    450                 455                 460

Lys Arg Lys His Ala
465
```

<210> 7
<211> 1410
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(1410)

<220>
<223> Description of Artificial Sequence: Bovine papillomavirus type 1 L2 open reading frame (humanized)

<220>
<223> Wild-type codons replaced with synonymous codons used at relatively high frequency by human genes

<400> 7

```
atg agc gcc cgc aag aga gtg aag cgc gcc agc gcc tac gac ctg tac   48
Met Ser Ala Arg Lys Arg Val Lys Arg Ala Ser Ala Tyr Asp Leu Tyr
  1               5                  10                  15

agg acc tgc aag cag gcc ggc aca tgt cca cca gat gtg atc cga aag   96
Arg Thr Cys Lys Gln Ala Gly Thr Cys Pro Pro Asp Val Ile Arg Lys
             20                  25                  30

gtg gag ggc gac acc atc gcc gac aag atc ctg aag ttc ggc ggc ctg   144
Val Glu Gly Asp Thr Ile Ala Asp Lys Ile Leu Lys Phe Gly Gly Leu
         35                  40                  45

gcc atc tac ctg ggc ggc ctg ggc atc gga aca tgg tct acc ggc agg   192
Ala Ile Tyr Leu Gly Gly Leu Gly Ile Gly Thr Trp Ser Thr Gly Arg
     50                  55                  60

gtg gcc gcc ggc ggc tca cca agg tac acc cca ctg cgc acc gcc ggc   240
Val Ala Ala Gly Gly Ser Pro Arg Tyr Thr Pro Leu Arg Thr Ala Gly
 65                  70                  75                  80

tcc acc tcc tcc ctg gcc tcc atc gga tcc aga gcc gtg acc gcc ggg   288
Ser Thr Ser Ser Leu Ala Ser Ile Gly Ser Arg Ala Val Thr Ala Gly
                 85                  90                  95

acc cgc ccc tcc atc ggc gcg ggc atc cct ctg gac acc ctg gaa act   336
Thr Arg Pro Ser Ile Gly Ala Gly Ile Pro Leu Asp Thr Leu Glu Thr
            100                 105                 110

ctt ggg gcc ctg cgc cct ggc gtg tac gag gac acc gtg ctg ccc gaa   384
Leu Gly Ala Leu Arg Pro Gly Val Tyr Glu Asp Thr Val Leu Pro Glu
        115                 120                 125

gcc cct gcc atc gtg acc cct gac gcc gtg cct gca gac tcc ggc ctg   432
Ala Pro Ala Ile Val Thr Pro Asp Ala Val Pro Ala Asp Ser Gly Leu
    130                 135                 140
```

```
gac gcc ctg tcc atc ggc aca gac tcc tcc acc gag acc ctg atc acc   480
Asp Ala Leu Ser Ile Gly Thr Asp Ser Ser Thr Glu Thr Leu Ile Thr
145                 150                 155                 160

ctg ctg gag cct gag ggc ccc gaa gac ata gcc gtg ctg gaa ctc cag   528
Leu Leu Glu Pro Glu Gly Pro Glu Asp Ile Ala Val Leu Glu Leu Gln
                165                 170                 175

ccc ctg gac cgc cca acc tgg cag gtg agc aat gct gtg cac cag tcc   576
Pro Leu Asp Arg Pro Thr Trp Gln Val Ser Asn Ala Val His Gln Ser
            180                 185                 190

tct gcc tac cac gcc cct ctc cag ctg caa tcc tcc atc gcc gag aca   624
Ser Ala Tyr His Ala Pro Leu Gln Leu Gln Ser Ser Ile Ala Glu Thr
        195                 200                 205

tct ggt tta gaa aat att ttt gta gga ggc tcg ggt tta ggg gat acc   672
Ser Gly Leu Glu Asn Ile Phe Val Gly Gly Ser Gly Leu Gly Asp Thr
    210                 215                 220

ggc ggc gag aac atc gag ctg acc tac ttc ggc tcc ccc cgc acc agc   720
Gly Gly Glu Asn Ile Glu Leu Thr Tyr Phe Gly Ser Pro Arg Thr Ser
225                 230                 235                 240

acc ccc cgc tcc atc gcc tcc aag tcc cgc ggc atc ctg aac tgg ttc   768
Thr Pro Arg Ser Ile Ala Ser Lys Ser Arg Gly Ile Leu Asn Trp Phe
                245                 250                 255

agc aag cgg tac tac acc cag gtg ccc acc gaa gat ccc gaa gtg ttc   816
Ser Lys Arg Tyr Tyr Thr Gln Val Pro Thr Glu Asp Pro Glu Val Phe
            260                 265                 270

tcc tcc cag acc ttc gcc aac ccc ctg tac gag gcc gag ccc gcc gtg   864
Ser Ser Gln Thr Phe Ala Asn Pro Leu Tyr Glu Ala Glu Pro Ala Val
        275                 280                 285

ctg aag ggc cct agc ggc cgc gtg ggc ctg tcc cag gtg tac aag cct   912
Leu Lys Gly Pro Ser Gly Arg Val Gly Leu Ser Gln Val Tyr Lys Pro
    290                 295                 300

gat acc ctg acc aca cgt agc ggc aca gag gtg ggc ccc cag ctg cat   960
Asp Thr Leu Thr Thr Arg Ser Gly Thr Glu Val Gly Pro Gln Leu His
305                 310                 315                 320

gtg agg tac tcc ctg tcc acc atc cat gag gat gtg gag gct atc ccc   1008
Val Arg Tyr Ser Leu Ser Thr Ile His Glu Asp Val Glu Ala Ile Pro
                325                 330                 335

tac acc gtg gat gag aac acc cag ggc ctg gcc ttc gtg ccc ctg cat   1056
Tyr Thr Val Asp Glu Asn Thr Gln Gly Leu Ala Phe Val Pro Leu His
            340                 345                 350

gag gag cag gcc ggc ttc gag gag atc gag ctc gac gat ttc agc gag   1104
Glu Glu Gln Ala Gly Phe Glu Glu Ile Glu Leu Asp Asp Phe Ser Glu
        355                 360                 365
```

```
acc cat cgc ctg ctg ccc cag aac acc tcc tcc acc ccc gtg ggc agc   1152
Thr His Arg Leu Leu Pro Gln Asn Thr Ser Ser Thr Pro Val Gly Ser
    370                 375                 380

ggc gtg cgc aga agc ctg atc cct acc cga gag ttc agc gcc acc cgg   1200
Gly Val Arg Arg Ser Leu Ile Pro Thr Arg Glu Phe Ser Ala Thr Arg
385                 390                 395                 400

cct acc ggc gtg gtg acc tac ggc tcc ccc gac acc tac tcc gct agc   1248
Pro Thr Gly Val Val Thr Tyr Gly Ser Pro Asp Thr Tyr Ser Ala Ser
                405                 410                 415

ccc gtg acc gac cct gat tct acc tct cct agc ctg gtg atc gac gac   1296
Pro Val Thr Asp Pro Asp Ser Thr Ser Pro Ser Leu Val Ile Asp Asp
            420                 425                 430

acc acc acc acc ccc atc atc atc atc gac ggc cac aca gtg gat ctg   1344
Thr Thr Thr Thr Pro Ile Ile Ile Ile Asp Gly His Thr Val Asp Leu
        435                 440                 445

tac agc agc aac tac acc ctg cat ccc tcc ctg ctg agg aag cgc aag   1392
Tyr Ser Ser Asn Tyr Thr Leu His Pro Ser Leu Leu Arg Lys Arg Lys
    450                 455                 460

aag cgc aag cat gcc taa                                           1410
Lys Arg Lys His Ala
465                 470
```

<210> 8
<211> 469
<212> PRT
<213> Artificial Sequence

<400> 8

```
Met Ser Ala Arg Lys Arg Val Lys Arg Ala Ser Ala Tyr Asp Leu Tyr
  1               5                  10                  15

Arg Thr Cys Lys Gln Ala Gly Thr Cys Pro Pro Asp Val Ile Arg Lys
             20                  25                  30

Val Glu Gly Asp Thr Ile Ala Asp Lys Ile Leu Lys Phe Gly Gly Leu
         35                  40                  45

Ala Ile Tyr Leu Gly Gly Leu Gly Ile Gly Thr Trp Ser Thr Gly Arg
     50                  55                  60

Val Ala Ala Gly Gly Ser Pro Arg Tyr Thr Pro Leu Arg Thr Ala Gly
 65                  70                  75                  80

Ser Thr Ser Ser Leu Ala Ser Ile Gly Ser Arg Ala Val Thr Ala Gly
                 85                  90                  95

Thr Arg Pro Ser Ile Gly Ala Gly Ile Pro Leu Asp Thr Leu Glu Thr
            100                 105                 110
```

```
Leu Gly Ala Leu Arg Pro Gly Val Tyr Glu Asp Thr Val Leu Pro Glu
        115                 120                 125

Ala Pro Ala Ile Val Thr Pro Asp Ala Val Pro Ala Asp Ser Gly Leu
    130                 135                 140

Asp Ala Leu Ser Ile Gly Thr Asp Ser Ser Thr Glu Thr Leu Ile Thr
145                 150                 155                 160

Leu Leu Glu Pro Glu Gly Pro Glu Asp Ile Ala Val Leu Glu Leu Gln
                165                 170                 175

Pro Leu Asp Arg Pro Thr Trp Gln Val Ser Asn Ala Val His Gln Ser
            180                 185                 190

Ser Ala Tyr His Ala Pro Leu Gln Leu Gln Ser Ser Ile Ala Glu Thr
        195                 200                 205

Ser Gly Leu Glu Asn Ile Phe Val Gly Gly Ser Gly Leu Gly Asp Thr
    210                 215                 220

Gly Gly Glu Asn Ile Glu Leu Thr Tyr Phe Gly Ser Pro Arg Thr Ser
225                 230                 235                 240

Thr Pro Arg Ser Ile Ala Ser Lys Ser Arg Gly Ile Leu Asn Trp Phe
                245                 250                 255

Ser Lys Arg Tyr Tyr Thr Gln Val Pro Thr Glu Asp Pro Glu Val Phe
            260                 265                 270

Ser Ser Gln Thr Phe Ala Asn Pro Leu Tyr Glu Ala Glu Pro Ala Val
        275                 280                 285

Leu Lys Gly Pro Ser Gly Arg Val Gly Leu Ser Gln Val Tyr Lys Pro
    290                 295                 300

Asp Thr Leu Thr Thr Arg Ser Gly Thr Glu Val Gly Pro Gln Leu His
305                 310                 315                 320

Val Arg Tyr Ser Leu Ser Thr Ile His Glu Asp Val Glu Ala Ile Pro
                325                 330                 335

Tyr Thr Val Asp Glu Asn Thr Gln Gly Leu Ala Phe Val Pro Leu His
            340                 345                 350

Glu Glu Gln Ala Gly Phe Glu Glu Ile Glu Leu Asp Asp Phe Ser Glu
        355                 360                 365

Thr His Arg Leu Leu Pro Gln Asn Thr Ser Ser Thr Pro Val Gly Ser
    370                 375                 380

Gly Val Arg Arg Ser Leu Ile Pro Thr Arg Glu Phe Ser Ala Thr Arg
385                 390                 395                 400

Pro Thr Gly Val Val Thr Tyr Gly Ser Pro Asp Thr Tyr Ser Ala Ser
                405                 410                 415
```

```
Pro Val Thr Asp Pro Asp Ser Thr Ser Pro Ser Leu Val Ile Asp Asp
            420                 425                 430

Thr Thr Thr Thr Pro Ile Ile Ile Ile Asp Gly His Thr Val Asp Leu
        435                 440                 445

Tyr Ser Ser Asn Tyr Thr Leu His Pro Ser Leu Leu Arg Lys Arg Lys
    450                 455                 460

Lys Arg Lys His Ala
465
```

<210> 9
<211> 717
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Aequorea victoria gfp gene (humanized)

<220>
<221> CDS
<222> (1)..(717)

<400> 9

```
atg agc aag ggc gag gaa ctg ttc act ggc gtg gtc cca att ctc gtg   48
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
  1               5                  10                  15

gaa ctg gat ggc gat gtg aat ggg cac aaa ttt tct gtc agc gga gag   96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
             20                  25                  30

ggt gaa ggt gat gcc aca tac gga aag ctc acc ctg aaa ttc atc tgc   144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
         35                  40                  45

acc act gga aag ctc cct gtg cca tgg cca aca ctg gtc act acc ttc   192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
     50                  55                  60

tct tat ggc gtg cag tgc ttt tcc aga tac cca gac cat atg aag cag   240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
 65                  70                  75                  80

cat gac ttt ttc aag agc gcc atg ccc gag ggc tat gtg cag gag aga   288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                 85                  90                  95

acc atc ttt ttc aaa gat gac ggg aac tac aag acc cgc gct gaa gtc   336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110

aag ttc gaa ggt gac acc ctg gtg aat aga atc gag ctg aag ggc att   384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
```

```
        115                 120                 125

gac ttt aag gag gat gga aac att ctc ggc cac aag ctg gaa tac aac   432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

tat aac tcc cac aat gtg tac atc atg gcc gac aag caa aag aat ggc   480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

atc aag gtc aac ttc aag atc aga cac aac att gag gat gga tcc gtg   528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

cag ctg gcc gac cat tat caa cag aac act cca atc ggc gac ggc cct   576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190

gtg ctc ctc cca gac aac cat tac ctg tcc acc cag tct gcc ctg tct   624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

aaa gat ccc aac gaa aag aga gac cac atg gtc ctg ctg gag ttt gtg   672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

acc gct gct ggg atc aca cat ggc atg gac gag ctg tac aag tga       717
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
```

<210> 10
<211> 238
<212> PRT
<213> Artificial Sequence

<400> 10

```
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
  1               5                  10                  15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
             20                  25                  30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
         35                  40                  45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
     50                  55                  60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
 65                  70                  75                  80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                 85                  90                  95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110
```

```
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
```

<210> 11
<211> 717
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(717)

<220>
<223> Description of Artificial Sequence: Synthetic gfp gene (Papillomavirusized)

<220>
<223> Codons of humanized gfp gene replaced with synonymous codons used at relatively high frequency by papillomavirus genes

<400> 11

```
atg agt aaa ggg gaa gaa cta ttt aca ggg gtg gtg cct ata cta gtg   48
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
  1               5                  10                  15

gaa cta gat ggg gat gtg aat ggg cac aaa ttt tct gtc agt ggg gaa   96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
             20                  25                  30

ggg gaa ggg gat gca aca tat ggg aaa cta aca cta aaa ttt ata tgc   144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
         35                  40                  45
```

```
aca aca ggg aaa cta cct gtg cca tgg cct aca cta gtg aca aca ttt   192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
     50                  55                  60

agt tat ggg gtg caa tgc ttt agt aga tat cct gat cat atg aaa caa   240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
 65                  70                  75                  80

cat gat ttt ttt aaa agt gca atg ccc gag ggg tat gtg caa gaa aga   288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                 85                  90                  95

aca ata ttt ttt aaa gat gat ggg aat tat aaa aca aga gca gaa gtc   336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110

aaa ttt gaa ggg gat aca cta gtg aat aga ata gag ctc aaa ggg ata   384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125

gat ttt aaa gaa gat ggg aat ata cta ggg cat aaa cta gaa tat aat   432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

tat aat agt cat aat gtg tat ata atg gca gat aaa caa aaa aat ggg   480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

ata aaa gtg aat ttt aaa ata ata aga cat ata gaa gat gga tcc gtg   528
Ile Lys Val Asn Phe Lys Ile Ile Arg His Ile Glu Asp Gly Ser Val
                165                 170                 175

caa cta gca gat cat tat caa caa aat aca cct ata ggg gat ggg cct   576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190

gtg cta cta cct gat aac cat tat cta agt aca caa agt gca cta agt   624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

aaa gat cct aat gaa aaa aga gat cat atg gtg cta ctc gag ttt gtg   672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

aca gca gca ggg ata aca cat ggg atg gat gaa cta tat aaa tga       717
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
```

<210> 12
<211> 238
<212> PRT
<213> Artificial Sequence

<400> 12

```
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
  1               5                  10                  15
```

```
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
             20                  25                  30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
         35                  40                  45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    . 50                  55                  60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
 65                  70                  75                  80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                 85                  90                  95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

Ile Lys Val Asn Phe Lys Ile Ile Arg His Ile Glu Asp Gly Ser Val
                165                 170                 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
```

<210> 13
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Ala(GCA)

<400> 13
taaggactgt aagactt 17

<210> 14
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Arg(CGA)

<400> 14
cgagccagcc aggagtc 17

<210> 15
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Asn(AAC)

<400> 15
ctagattggc aggaatt 17

<210> 16
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Asp(GAC)

<400> 16
taagatatat agattat 17

<210> 17
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Cys(TGC)

<400> 17
aagtcttagt agagatt 17

<210> 18
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Glu(GAA)

<400> 18
tatttctaca cagcatt 17

<210> 19
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Gln(CAA)

<400> 19
ctaggacaat aggaatt 17

<210> 20
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Gly(GGA)

<400> 20
tactctcttc tgggttt 17

<210> 21
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for His(CAC)

<400> 21
tgccgtgact cggattc 17

<210> 22
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Ile(ATC)

<400> 22

tagaaataag agggctt 17

<210> 23
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Leu(CTA)

<400> 23
tacttttatt tggattt 17

<210> 24
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Leu(CTT)

<400> 24
tattagggag aggattt 17

<210> 25
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Lys(AAA)

<400> 25
tcactatgga gatttta 17

<210> 26
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Lys(AAG)

<400> 26
cgcccaacgt ggggctc 17

<210> 27
<211> 17

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Met (elong)

<400> 27
tagtacggga aggattt 17

<210> 28
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Phe(TTC)

<400> 28
tgtttatggg atacaat 17

<210> 29
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Pro(CCA)

<400> 29
tcaagaagaa ggagcta 17

<210> 30
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Pro(CCI)

<400> 30
gggctcgtcc gggattt 17

<210> 31
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:

Oligonucleotide specific for Ser(AGC)

<400> 31
ataagaaagg aagatcg 17

<210> 32
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Thr(ACA)

<400> 32
tgtcttgaga agagaag 17

<210> 33
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Tyr(TAC)

<400> 33
tggtaaaaag aggattt 17

<210> 34
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide specific for Val(GTA)

<400> 34
tcagagtgtt cattggt 17

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00530

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/37; C07K 14/025

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: as above

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN (CA; Medline): codon usage/CT; gene expression /CT
ORBIT (WPAT): codon () use or codon () usage or codon() bias

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	AU-43556/97 (THE GENERAL HOSPITAL CORPORATION) 26 March 1998 Claim 1-28	1-25
X,Y	AU-35099/95 (THE GENERAL HOSPITAL CORPORATION) 28 March 1996 Claims 1-16	1-25
P,X	AU-17502/97 (UNIVERSITY OF FLORIDA RESEARCH FOUNDATION) 24 July 1997 pp 2-12; pp 21-74	1-25

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
19 August 1998

Date of mailing of the international search report
-2 SEP 1998

Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200
WODEN ACT 2606
AUSTRALIA
Facsimile No.: (02) 6285 3929

Authorized officer

MADHU K. JOGIA

Telephone No.: (02) 6283 2512

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	AU-74467/96 (DOWELANCO, USA) 17 April 1997 Claims 1-6	1-25
X	J. VIROL, Vol. 70, pp 4646-4654 (1996) ZOLOTUKHIN et al A "humanized" green fluorescent protein CDNA adapted for high level expression in mammalian cells.	1-25

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 98/00530

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
AU	43556/97	WO	9812207				
AU	35099/95	CA	2200342	EP	781329	US	5786464
		WO	9609378				
WO	9726333	AU	17502/97				
WO	9713402	AU	74467/96				

END OF ANNEX

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.